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7870

Induced Resistance to Transmissible Leukemia in Mice.

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MacDowell, Taylor, and Potter[1] present evidence that mice which are naturally susceptible to transplantable leukemia may be rendered insusceptible by treatment with small numbers of leukemic cells. Bashford[2] and others have shown that mice which are naturally susceptible to a transplantable tumor may be immunized by treatment with suspensions of normal mouse cells. The experiments suggested the possibility of rendering susceptible mice refractory to transplantable leukemia by treatment with normal mouse cells.

A strain of transplantable lymphatic leukemia and a group of susceptible mice (AR strain) were obtained through the kindness of Dr. Jacob Furth of the Cornell Medical College. The mouse strain is one which is not abnormally prone to develop spontaneous leukemia. Suspensions of the spleens and lymph nodes of leukemic mice have been passed to normal susceptible mice on an average of

[1] MacDowell, E. C., Taylor, M. J., and Potter, J. S., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 84.

[2] Bashford, E. F., Trans. XVIIth Internat. Cong. Med., London, 1913, Subsection III, a29.

TABLE I.

Experiment I. Induced resistance with cells of a different strain.

No. of Mice	Treatments with normal cells	Interval days	Route	Interval before leukemia transplantation days	No. injected with leukemia cells	Died of Leukemia	Survived	% resistant
11	4	1	Intravenous and Intraperitoneal	7	11	3	8	72.
8	4	1	"	12	8	0	8	100.
9	4	1	"	30	9	1	8	89.
18	Direct controls			—	18	18	0	0
	Experiment II. Induced resistance with cells of the same strain.							
14	3	2	Intravenous	2	14	3	11	78.5
15	3	2	"	7	13	4	8	53.5
12	Direct controls					12	0	0

once in 6 days. A standard suspension containing about 100,000 cells per mm.^{3} has been inoculated intravenously in 0.2 cc. amounts. Typical leukemia has resulted in 353 of 360 passage animals, a percentage susceptibility of 98.1.

In Experiment I the effect of treating the susceptible mice with normal mouse cells of another strain which was not susceptible to transplantable leukemia was tested. Twenty-eight susceptible animals were treated on 3 successive days by the intravenous injection of 0.2 cc. of a suspension of minced spleen and lymph-node tissue filtered through cotton from normal mice of the Rockefeller Institute strain. On the fourth day an intraperitoneal injection of 0.5 cc. of similarly prepared material was made. After an interval of 7 days, 11 mice of the group were injected intravenously with 0.2 cc. of a standard suspension of spleen and lymph-node cells from a leukemic mouse of the same strain. After 12 days 8 more, and after 30 days the remaining 9 were similarly treated. Of the first group, which received leukemic cells 7 days after the prophylactic treatment, 3 animals developed fatal leukemia and 8 survived, a survival percentage of 72.5. Of the second group, inoculated with leukemia 12 days after immunization, all survived. Of the 9 animals of the third group, injected with leukemic cells 30 days after immunization, 8 survived, a percentage of 89.

In a second set of experiments 29 susceptible mice were treated 3 times at 2-day intervals by the intravenous injection of 0.2 cm. of an emulsion of minced spleen and lymph-node tissue of normal mice of the same strain. Two animals died of intercurrent disease. Two days following the last immunizing treatment 0.2 cc. of a standard suspension of leukemic cells was inoculated intravenously in 14 animals. Seven days following the last immunizing treatment the remaining 13 mice were similarly treated. Of the first group 3 mice died with typical leukemia and the remainder survived. Of the second group one died of intercurrent disease without leukemia, 4 died with leukemia, and the remainder survived, a survival percentage of 53.5.

In the 5 experiments reported the average survival percentage was 78.6 as compared to failure of any of the controls to survive. Moreover, in routine passages of the same leukemia strain over a period of 8 months only 7 animals survived out of 360 inoculated, a survival percentage of 1.9. Although no conclusions can be drawn as to the effect of the route or cell strain used in the prophylactic treatment, the fact appears to be established that mice normally susceptible to transplantable leukemia can be rendered resistant by the intravenous injection of normal spleen and lymph-node suspensions.

7871 C*

Effect of Low Pressure on the Blood Picture of Necturus Maculosus.

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This problem was undertaken for three reasons. (1) There are no well controlled experiments, so far as I know, in which an aquatic form has been subjected to a constant low pressure and examined for changes in its blood picture. Necturus was chosen as the experimental animal because of the readily available source of blood in the afferent branchial arteries of the external gills and because the normal blood picture has been thoroughly worked out by Dawson.[1] (2) Experiments such as those described below supply a method for determining the nature of the red cell ancestors in Necturus by causing them to leave the erythrocytopoietic loci and enter the circulating blood. (3) It is interesting to compare the intensity and duration of the stimulus required to send out erythroid cells into the blood stream of an aquatic form with the intensity and duration necessary to evoke an analogous response in mammals (*i. e.*, increase the reticulocyte and total red cell count).

Groups of Necturi were placed in jars of water and subjected to a constant pressure of 330 mm. Hg. in a specially devised low pressure chamber for varying periods up to 9 weeks of continuous exposure. (For the construction of the apparatus, see Dubin.[2]) The animals were removed once a day for about an hour, and the water in which they were kept was replaced by fresh water. All animals, including the controls, were fed live earthworms twice weekly. In order to minimize the effects of hemorrhage, the small quantity of blood necessary for total counts and 2 smears was drawn on 2 occasions only, once, before the animals were placed in the tank, and later, when they were examined to determine the effects of the reduced pressure. Because of the relatively small red cell count in Necturus (about 42,000 per mm^3. as determined in 28 normal animals), total red cell counts were made by drawing blood from the afferent branchial artery up to the 0.1 mark in a white cell pipette, diluting with Hayem's solution or 0.65% saline containing methylene blue, and counting the cells on 800 squares of a Levy-Hausser counting chamber. By this method the maximum error incurred in counting

* C represents a complete, P a preliminary manuscript.

1 Dawson, Alden B., *J. Morph.*, 1933, **55**, 349.

2 Dubin, Max, *Quart. J. Exp. Physiol.*, 1934, **24**, 31.

red cells in consecutive samples drawn from trial animals was ±6%. White cell counts were made on the same preparation (since they are easily distinguishable from the reds) except that the cells on an area equivalent to 4,000 squares were counted. In making the white cell counts by this method, it is difficult to distinguish between haemocytoblasts and true leucocytic elements, and so both were included in the total count. The average in 16 animals was found to be 440 per mm^3. with a maximum error of ±20%. These counts must be made immediately after the extraction of the blood, for the cells sediment rapidly in the pipette and then tend to disintegrate.

No significant changes in the circulating blood picture were noted until about the 4th or 5th week, when the total red cell count began to show a significant increase. By the end of the 6th week the presence of considerable numbers of red cell progenitors (*i. e.*, haemocytoblasts, proerythroblasts, and erythroblasts) were observed in dry smears stained with Wright's or Pappenheim's stain. The 4 animals examined at the end of the 7th week of exposure showed a 25 to 35% increase in red cell count (average before exposure, 44,000 per mm^3.; average after exposure, 58,000 per mm^3.). Autopsies performed at this time showed that the spleens were no longer dark red (as in the controls), but a pale pink. The white cell counts of these animals at this period also showed a significant increase (average before exposure, 350 per mm^3.; average after exposure, 1520 per mm^3.) This increase was due almost entirely to the increased number of haemocytoblasts observable in the stained dry smears. The thrombocytes also increased in number. The neutrophile, eosinophile, and basophile numbers, however, did not alter significantly.

The red cell counts of the animals examined after 9 weeks of exposure did not show as great percentage increases as did those of the 7-week animals. Neither were the differentiating elements, the haemocytoblasts, proerythroblasts, and erythroblasts as numerous. In fact, it appears that the maximum reaction was reached at the end of the 7th week of exposure and then tended to diminish in intensity with continued exposure. Unpublished data show that rabbits react in the same way to low pressures except that the time taken to produce a response is considerably shorter. Thus, in 3 rabbits exposed to a pressure of 350 mm. Hg. the red cell count reached a maximum in from 7 to 10 days, and then began to fall despite the continued application of the stimulus.

Control animals examined at frequent intervals during the entire

course of the experiments showed none of the changes described above.

The definitive lymphocytes which Jordan[3] has described as occurring in the circulating blood of *Proteus anguineus* (in a prolonged state of inanition) and which Dawson[1] has noted in the blood of *Necturus maculosus* beginning with about the 12th day of immersion in water containing lead were not observed in Necturus even after the 9th week of exposure to the low pressure. The absence of these cells is not necessarily opposed to Dawson's claim that they act as erythroid progenitors when "the demand for new cells is excessive and prolonged". The increased demands in these experiments (due most likely to the decreased oxygen tension at the low pressure employed) are apparently adequately met by an outpouring from the spleen, first of immature red cells which divide by mitosis in the blood stream, and then of haemocytoblasts which proliferate and differentiate intravascularly. The demands do not become great enough to result in a release of lymphocytes from the haemocytopoietic loci.

It is interesting to note that Charipper[4] has shown that the quantity of thyroid extract necessary to stimulate the granulocytopoietic centers is much greater in Necturus than in mammals, and that the maximum point of the response (*i. e.*, the greatest deflection of the polynuclear count to the left) is reached considerably later. The above results demonstrate, in still another way, the sluggishness of the response of Necturus when compared with the response in mammals. It is only after Necturus has been kept for about 5 weeks at the pressure of 330 mm. Hg. that a response is evoked in the form of an outpouring of erythrogenic elements; Dubin,[2] on the other hand, has observed that rabbits need only to be subjected to a pressure of 411 mm. Hg. for 5 days to show a 7 to 10% increase in reticulocytes and a 30 to 40% increase in total red cell count.

[3] Jordan, H. E., *Am. J. Anat.*, 1932, **51**, 215.

[4] Charipper, H. A., *Quart. J. Exp. Physiol.*, 1928, **19**, 109.

7872 P

Inhibition of Leucogenic Activity in the Rabbit by Certain Cyclic Compounds.

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The leucopoietic apparatus of the rabbit under ordinary circumstances is an organ in a delicate state of equilibrium and the threshold for leucopoietic stimuli is low. It has been shown[1] that the intravenous injection of even a small quantity of distilled water may be capable of producing a stimulation characterized by a transient leucocytosis which is the result of a true bone marrow reaction, for it is accompanied by a sudden increase in the number of juvenile cells in the peripheral circulation. The more potent stimulating agents will produce a more profound reaction. Nucleic acid, or sodium nucleinate, are examples of such potent stimulating agents and the parenteral introduction of either of these substances into a normal rabbit will invariably be followed by a transient leucocytosis which is accompanied by a marked increase in the percentage of juvenile cells in the peripheral circulation.

During the course of an investigation into the action of certain cyclic compounds on the haematopoietic tissues, it was observed that the capability of the leucogenic tissues to respond to normal stimuli was lost long before any change could be observed in the cellular constituents of the peripheral circulation. These compounds which include amidopyrin, antipyrin, a-dinitrophenol, phenylhydrazine hydrochloride, catechol and o-quinone, have been described as possible etiological agents in the production of human agranulocytic angina.[2, 3, 4]

With the exception of the reaction given to a-dinitrophenol, the reaction to amidopyrin may be regarded as typical of the reactions to the entire group; the quantities of these substances which were required to inhibit the irritability of the leucogenic tissues varied greatly, phenylhydrazine being the most potent agent and antipyrin the least active.

For the sake of brevity, only one set of results is presented, but the action of each substance was investigated on a group of 8

1 Climenko, D. R., *Quart. J. Exp. Physiol.*, 1930, **20**, 369.

2 Kracke, R. R., and Parker, F. P., *J. Lab. Clin. Med.*, 1934, **19**, 799.

3 Silver, S., *J. Am. Med. Assn.*, 1934, **102**, 1213.

4 Madison, F. W., and Squier, T. L., *J. Am. Med. Assn.*, 1934, **102**, 755.

experimental animals with a control animal in each group. The results show a high degree of consistency and any one animal may be regarded as being typical of its group.

Rabbit 5 of Group I showed the following steady state

Date	r b c	Hb	w b c	% granulocytes	Polynuclear Count I	II	III	IV	V	Weighted Mean
9/20	6,200,000	75	9,200	28	23	33	29	12	3	2.39
9/21	6,200,000	75	8,100	30	22	33	31	11	3	2.40
9/22	6,400,000	75	10,400	28	22	31	30	12	5	2.47

Following the intramuscular injection of 5 mg./kg. of nucleic acid, the following results were obtained:

Hours after injection	r b c	Hb	w b c	% granulocytes	Polynuclear Count I	II	III	IV	V	Weighted Mean
0	6,400,000	75	8,200	29	24	32	30	12	2	2.36
1	———	—	8,800	31	30	38	23	8	1	2.12
2	———	—	14,200	37	41	28	21	10	0	2.00
4	———	—	15,100	34	42	28	18	12	0	2.00
8	6,300,000	74	10,600	34	40	29	20	11	0	2.02
24	———	—	8,600	36	31	39	21	9	0	2.17
48	6,280,000	75	7,800	32	27	43	23	7	0	2.10
96	6,100,000	75	8,000	27	25	33	32	9	1	2.28
240	6,200,000	75	6,800	29	21	32	30	14	3	2.46

At the end of this period of 10 days this animal received amidopyrin (0.2 gm./kg./day) orally for a period of 18 days. At the end of this time an intramuscular injection of 5 mg./kg. of nucleic acid failed to evoke the normal response as may be observed by an examination of the following data. The steady state for this animal was:

Date	r b c	Hb	w b c	% granulocytes	Polynuclear Count I	II	III	IV	V	Weighted Mean
10/22	5,800,000	79	9,200	28	25	35	27	9	4	2.32
10/23	6,050,000	75	8,600	31	24	36	24	11	5	2.32
10/25	5,900,000	75	11,400	27	27	34	28	9	2	2.25

The nucleic acid was injected and the following results were obtained:

Hours after injection	r b c	Hb	w b c	% granulocytes	Polynuclear Count I	II	III	IV	V	Weighted Mean
0	6,100,000	74	8,200	27	28	37	27	8	2	2.25
1	———	—	6,800	26	28	36	26	8	4	2.30
2	———	—	7,200	27	26	38	25	9	2	2.23
4	———	—	7,600	28	27	33	27	10	3	2.29
8	———	—	8,600	30	26	34	28	9	3	2.31
24	6,300,000	71	8,400	29	25	36	24	11	4	2.33
48	6,000,000	73	7,600	30	29	32	25	12	2	2.26
96	6,050,000	73	6,800	27	26	35	27	9	3	2.28
240	5,850,000	76	9,400	29	24	34	28	10	4	2.36

At the same time an animal that had been used as a control for this group responded to the second injection of nucleic acid in the same manner as it did to the original injection which had been given 28 days earlier. Similar inhibition was obtained after the administration of antipyrin, phenylhydrazine hydrochloride, o-quinone and catechol. The reaction to a-dinitrophenol differed slightly in that the quantity (20 mg./kg.) which was required to produce the inhibition of the leucogenic tissues, produced a preliminary stimulation which was characterized by an increase in the percentage of juvenile cells in the circulation without a concomitant leucocytosis. After the drug had been administered for a few days this reaction was lost and the proportions of the cellular elements in the peripheral circulation returned to normal. Shortly after this return to normal, the administration of nucleic acid failed to evoke a response.

The bone marrows of the experimental animals showed a marked degree of hyperplasia, with an increase in the number of primitive haemocytoblasts.

7873 C

The Influence of Sodium Thioglycollate on the Glycolytic Enzyme System of Muscle Extract.

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It has been suggested by Lipmann[1] that the enzyme (or one component of the enzyme system) of muscle extract which is capable of converting glycogen into lactic acid is reversibly oxidizable and reducible and is active as an enzyme only in its reduced state. This hypothesis was seemingly confirmed by Michaelis and Runnström.[2] The evidence presented by the latter authors for the reactivation of a muscle extract which had become partially or completely inactive by standing exposed to the air was as follows. If such an extract was treated with a neutralized solution of thioglycollic acid and then subjected to a manometric experiment, with the addition of sodium bicarbonate and a suitable partial pressure of CO_2, a definite and sus-

[1] Lipmann, F., *Biochem. Z.*, 1933, **265**, 133.

[2] Michaelis, L., and Runnström, J., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 343.

tained positive pressure was recorded on the manometer. If the thioglycollate was replaced by water a much smaller pressure or no pressure was recorded. This evidence was strengthened by the finding of a small but definite increase in lactic acid in the thioglycollate experiment as compared with the control.

Certain difficulties in preparing the mixtures containing thioglycollate for the application of the Friedemann, Cotonio and Shaffer[3] method for lactic acid were mentioned in the earlier paper, but it was believed that they had been overcome. We have now to report that this increase in lactic acid cannot be confirmed.

The method used by us is as follows: The contents of a respiration vessel amounting to about 2.5 cc.* is diluted to 20.0 cc. with H_2O, 10.0 cc. of a 20% $CuSO_4$ solution is added gradually with constant shaking, and 10.0 cc. of a 10% suspension of calcium hydroxide is then added. After thorough mixing the solution should be definitely alkaline to litmus. The volume is made up to 50.0 cc. with water and it is then allowed to stand for 10-18 hours. Upon filtering, from 28.0 to 33.0 cc. of clear filtrate are obtained. This is sufficiently free from protein that it gives no precipitate with sulfosalicylic acid and gives a negative Molisch test for carbohydrate.† This filtrate is used directly for the lactic acid determination. It shows no foaming and remains entirely clear so that the end point of the permanganate addition, the separation of MnO_2, is clearly discernible. The end point of the subsequent iodine titration is as sharp as with a determination on pure lactic acid.

By the use of this method we have found that the positive pressure developed in excess of the control in a manometric experiment with the addition of thioglycollate is not due to, or accompanied by, the formation of lactic acid. This positive pressure, which is readily

[3] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335. Wendel, W. B., *Ibid.*, 1933, **102**, 47.

* This 2.5 cc. contains 1.8 cc. of the muscle extract,[4] 0.2 cc. of a 5% solution of coenzyme,[5] sufficient $NaHCO_3$ to make the final concentration 0.025 m, (a gas mixture of 5% CO_2 and 95% N_2 was used), sufficient glycogen to give a final concentration of 0.2% and the thioglycollate (usually 0.3 cc. of a freshly neutralized 1.0 m. solution of sodium thioglycollate).

[4] Meyerhof, O., *Biochem. Z.*, 1926, **178**, 395.

[5] Lohmann, K., *Biochem. Z.*, 1931, **233**, 460.

† This is essentially the method used by Van Slyke[6] for the removal of sugars. With our material, under these conditions, it also removes the protein. A similar observation has been made and utilized by Elliott and Schroeder.[7]

[6] Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chem., Vol. II, p. 626.

[7] Elliott, K. A. C., and Schroeder, E. F., *Biochem. J.*, 1934, **28**, 1920.

obtained, is, according to our experience, due to the thioglycollate itself. An equally great, or even greater pressure, which continues for some hours at a decreasing rate, is obtained if the thioglycollate is added to pure sodium bicarbonate (*e. g.* 0.3 cc. of a 1.0 m. solution gives a pressure equal to 275 cmm. in 2 hours at 37°). The rate at which the pressure develops varies considerably with temperature. We have carried out experiments at 20°, 25°, 30°, and 37°. The results differ only quantitatively, being greater at the higher temperatures. This pressure is probably due to the fact that the thioglycollic acid contains one or more anhydrides which are slowly hydrolyzed during the experiment. This is in agreement with the finding that if an approximately 1.0 m. solution of our thioglycollic acid, which had been distilled 3 weeks previously, was refluxed for 2 hours, with volume control to see that no water was lost, it increased its titratable acidity by 14.3% as judged by titrating with NaOH using methyl red as indicator. This solution then developed much less pressure in a manometric experiment.

On the basis of the above findings we believe it is no longer justifiable to speak of a regeneration of this inactivated enzyme system by thioglycollate. We herewith withdraw the statement, and any conclusions based on the statement, that the inactivated glycolytic enzyme system of muscle can be regenerated by thioglycollate.

7874 P

Effect of Certain Agents on Cochlear Effect and Hearing.*

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There has been some question as to whether the Wever and Bray[1] phenomenon will prove to be an accurate tool for the study of hearing in animals. These authors showed that electrical currents could be picked up from the eighth nerve of the cat with a suitable amplifier. With telephone receiver or cathode ray oscillograph such cur-

* The work was made possible by grants from the Research Council of the American Otological Society and the Hayden-Coakley Fund.

1 Wever, E. G., and Bray, C. W., *J. Exp. Psychol.*, 1930, **5**, 373.

rents are found to have fundamentally the same frequency as the sound producing them; that is, if the 256 tuning fork is sounded near the ear of a cat properly hooked up, the sound of the fork is transmitted by the cochlea of the animal and can be picked up by an amplifier in another room and can be converted into sound or a graphic sine wave of 256 double vibrations. A similar phenomenon is obtained if the primary electrode is placed on or near the round window—this is called "the cochlear effect".

Several studies[2, 3, 4] have reported results tending to show that the cochlear effect may not correlate with hearing, while others[5, 6] have recently reported evidence of such correlation. On the other hand, from the point of view of an attempt to explain the cochlear mechanism, there have been various experiments to eliminate portions of the auditory response by stimulation[5, 7, 8] and by drugs.[9]

Hallpike and Rawdon-Smith[10] have recently reported that a crystal of sodium chloride in the niche of the round window abolishes the cochlear effect. In a series of experiments on the cause of nerve deafness, we have tried several other substances on the round window. We find that glycerin and solutions, as well as crystals of sodium chloride, calcium chloride and quinine dihydrochloride all produce abolition of the cochlear effect if left in the niche of the round window long enough. With low concentrations they produce a progressive diminution of the high tones and a smaller diminution of the low tones, both of which are proportional to the concentration of the solution used and the length of time they are in contact with the round window. With glycerin we have observed recovery in one animal after 5 days, but with electrolytes we have observed no recovery to date. We have been unable to produce significant diminution of the cochlear effect, within the limits of our apparatus, by the placing of crystals of glucose or dextrose on the round window; nor is there any appreciable effect with distilled water.

In the apparatus used by us, the primary electrode was a shielded

[2] Davis, H., Derbyshire, A. J., Lurie, M. H., and Saul, L. J., *Am. J. Physiol.*, 1934, **107**, 311.

[3] Guttmann, J., and Barrera, S. E., *Am. J. Physiol.*, 1934, **109**, 704.

[4] Culler, E., Finch, G., and Girden, E. S., *Science*, 1933, **78**, 269.

[5] Davis, H., Derbyshire, A. J., Kemp, E. H., Lurie, M. H., and Upton, M., *Science*, 1935, **81**, 101.

[6] Hallpike, C. S., and Rawdon-Smith, A. F., *J. Physiol.*, 1934, **83**, 243.

[7] Wever, E. G., Bray, C. W., and Horton, G. P., *Science*, 1934, **80**, 18-19.

[8] Finch, G., and Culler, E., *Science*, 1934, **80**, 41.

[9] Adrian, E. D., Bronk, D. W., and Phillips, G., *J. Physiol.*, 1931, **73**, 2P.

[10] Hallpike, C. S., and Rawdon-Smith, A. F., *J. Physiol.*, 1934, **81**, 395.

copper wire on which was a small cotton plug kept wet with normal saline, placed on or near the round window. The indifferent electrode was a zinc-plated test clip on the exposed neck muscles of the animal. The amplifier was a 3-stage, capacitance-resistance coupled one with high resistance input and a gain control in the second and third stages. Amplification was adjusted to bring the cochlear response to audible threshold with the use of head phones. The sounds used were an ordinary harmonica, a Galton whistle, and in some of the experiments, a shielded audiometer. In all cases, both ears were exposed through the bullae in the neck and tested alternately. The experimental chemical was washed out of the middle ear with normal saline and the cavities cleaned of blood clots and excess fluid before testing. The control ear was always similarly cleaned.

To check the correlation of cochlear effect with hearing, young dogs were conditioned to sounds from the audiometer according to a modified method of Culler.[4] They were then subjected to the operation. Salt was placed on the round window on one side until the electrical responses were diminished or absent. When they had sufficiently recovered from the anesthetic, they were again tested with the sounds to which they had previously been conditioned. They showed a marked rise in auditory threshold (especially for the high notes) in the ear in which the cochlear response had been diminished by the placing of salt upon the round window. These dogs were operated on both sides, showed no subsequent middle ear infection, and no essential diminution on the side in which no chemical was placed on the round window. The fact that the high notes (which are supposed to act upon that portion of the basilar membrane nearest the round window) are effected more quickly and more severely than the low notes adds to the evidence for a "place" theory of hearing. The parallel loss of the electrical response and loss of hearing of dogs, tested by conditioning to the audiometer, constitutes another step in the correlation of hearing and the cochlear effect.

7875 P

Serial Transmission of Virus of Infectious Papillomatosis in Domestic Rabbits.

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It was reported[1] that the virus of infectious papillomatosis produced warts which were readily transmissible in its natural host, the wild cottontail rabbit. However, warts which it regularly induced in domestic rabbits were not transmissible serially in either wild or domestic rabbits. A similar situation as regards the transmission of Rous Sarcoma 1 to turkeys, guinea fowls, and pheasants has been described.[2, 3]

The purpose of the present paper is to report the successful transmission of the virus of infectious papillomatosis serially in domestic rabbits. Warts from domestic rabbits, infected with papilloma virus of wild rabbit origin in the usual fashion,[1] were removed at various intervals, ground with sand, suspended in physiological saline and the resulting suspension used in attempts to infect other domestic rabbits. The technique of infection was the same that had been used unsuccessfully in earlier experiments. Thirty-two attempts to carry the virus beyond its first domestic rabbit passage have been made since the 26 fruitless efforts first reported.[1] Of these, 13 were successful, the domestic rabbits of the second serial passage developing one or more papillomas over the area of skin inoculated. Efforts to maintain the virus in domestic rabbits beyond its second serial passage were made in 7 instances and, of these, 4 were successful. Two series were carried through their fifth domestic rabbit passage and then discontinued. The remaining 2 series are still being passed; one has reached its 6th and the other its 10th domestic rabbit passage.

The individual papillomas developing in rabbits infected with the domestic rabbit-passaged virus are identical in all respects with those resulting following similar infection with virus obtained from wild rabbit warts. The incubation period following infection with the domestic rabbit virus, however, ranges from 13 to 30 days as contrasted to a 6 to 12-day incubation period following infection with

[1] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

[2] des Ligneris, M. J. A., *Am. J. Cancer*, 1932, **16**, 307.

[3] Andrewes, C. H., *J. Path. and Bact.*, 1932, **35**, 407; 1933, **37**, 17.

wild rabbit virus. Also the extensive confluent folded mass of papillomatous tissue resulting from infection with wild rabbit virus is seldom seen even following a heavy infection with the domestic rabbit virus; rather the papillomata induced by the latter virus tend to be more isolated and discrete over the area of skin inoculated. Similar isolated papillomata developing after a prolonged incubation period result when rabbits are infected with very dilute wild rabbit virus. These 2 differences are thus probably quantitative and merely indicate that the wild rabbit papillomata contain a higher concentration of available virus than do those from domestic rabbits.

No fact has been yet brought to light to explain why, in certain instances, domestic rabbit papillomata prove transmissible in series and in other instances non-transmissible. That the degree of maturity of the warts in domestic rabbits at the time that attempts were made to transmit them was not a determining factor is indicated by the fact that the ages of those that proved transmissible ranged from 7 to 377 days, while warts ranging in age from 1 to 116 days have proven non-transmissible. The breed of rabbit used seems also to be immaterial. Preliminary experiments suggest that the age of the rabbit may be of some importance, for warts taken from rabbits over 2 years old have proven serially transmissible more frequently than warts taken from rabbits 2 to 4 months old. It seems likely that, in determining whether or not a domestic rabbit wart will prove transmissible at least 2 factors are involved; the concentration of the inciting agent in the papillomata used as the source of infection, and the natural threshold of resistance, to the virus, of the animal to be infected. A successful passage of the disease in series from one domestic rabbit to the next, on this basis, can be visualized as the result of a fortunate and unpredictable coincidence in which the papillomata of the rabbit serving as the source of infection are especially rich in virus and the natural threshold of resistance, to the virus of the animal to be infected is unusually low. Factors determining these 2 conditions are not yet understood. Experiments with the domestic rabbit-passaged virus make it appear likely that, after 3 or 4 serial passages through domestic rabbits, transmission from animal to animal is more easily effected and that, in fact, an actual adaptation to the new host is in process. But even during these serial passages a rabbit is occasionally encountered whose warts fail to infect. In such instances it is necessary to revert to glycerol-stored material from an earlier passage in order to carry the series on.

This is a preliminary report published only to correct the con-

ception created by an earlier paper[1] that the virus of infectious papillomatosis was never serially transmissible in domestic rabbits. Experiments attempting to determine accurately the conditions necessary for the regular serial transmission in domestic rabbits are in progress.

7876 C

Attempts to Produce Poliomyelitis in Refractory Laboratory Animals.*

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Since the successful transmission to monkeys of the virus of poliomyelitis by Landsteiner and Popper,[1] many attempts have been made to produce the disease in the other laboratory animals. This work has been adequately reviewed by Shaughnessy *et al.*[2] and Harrington.[3] Experiments concerned with transmission of the virus to any animal other than the monkey have given only negative results. In this work, the passage and multiplication of the virus of poliomyelitis was attempted in mice, rats, guinea pigs and rabbits. It was thought possible to overcome the factors which make these animals naturally resistant to the virus, by using very young animals, by lowering their resistance and by passing the virus serially in the hope of adapting it to the new host.

First, to determine the time of survival of the virus in the brains of these animals, they were injected intracerebrally and after various intervals of time, the site of inoculation was removed, emulsified and injected into monkeys to determine the presence of virus. Rabbits received 0.3 cc., guinea pigs 0.2 cc., rats 0.1 cc., and mice 0.03 cc. of virus suspension. Table I indicates the time of removal of

* This work was aided by grants from the New York and Rockefeller Foundations and a fund from Mr. Jeremiah Milbank.

1 Landsteiner, K., and Popper, E., *Z. f. Immunitätsforsch. und exp. Therap.*, 1909, **2**, 377.

2 Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B., *J. Prev. Med.*, 1930, **4**, 59, 89.

3 Poliomyelitis, International Committee, 1932, p. 101. Williams and Wilkins, Baltimore, 1932.

brain tissue and the subsequent reaction in monkeys inoculated with this tissue. The results are summarized in Table I.

TABLE I.

Animal	Amount of virus suspension injected	Interval between inoculation of virus and removal of brain days	Result of injection of brain tissue into monkeys.
Rat (6 weeks old)	0.1 cc. of 10% suspension, intracerebrally	5	no paralysis
		3	" "
		2	" "
Guinea Pig (2 days old)	0.1 cc. of 20% suspension, intracerebrally, and 0.3 cc. intraperitoneally	4	" "
		3	" "
		2	" "
Guinea Pig (adult)	0.2 cc. of 20% suspension intracerebrally and 0.3 cc. intraperitoneally	3	" "
		2	" "
Rabbit (adult)	0.2 cc. of 20% suspension intracerebrally	5	" "
		3	" "
		2	paralysis 11 days
Mouse (adult)	0.03 cc. of 20% suspension intracerebrally	5	no paralysis
		3	paralysis 7 days

It appears that the mouse is the best of the refractory animals for use in this work, since the virus survived in its brain for from 3 to 5 days. In the rabbit brain the virus is demonstrable 2 to 3 days after injection.

Attempts to Adapt the Virus to Mice and Rats by Serial Passage. White, male rats, six weeks old, received combined intracerebral, intraperitoneal and subcutaneous injections of 0.15 cc., 0.5 cc., and 0.3 cc., respectively, given 2 to 3 times during 24 hours. Six hours after the last injection, the animals were sacrificed, the sites of inoculation including part of the peritoneal epithelium were removed and suspended in distilled water. To this material was added an equal amount of active virus and the mixture was injected into another rat by all 3 routes, 2 to 3 times during 24 hours. Further serial passage was then carried out in this manner for 16 passages, using animals of the same age. The rat on the 16th passage received 1 to 3 injections daily over a period of 13 days, and after a 2-day rest period was again injected on the 16th and 17th days in the hope that the brain trauma increased its susceptibility. At no time did this animal or those of earlier passages show any evidence of poliomyelitis.

With mice, the same technic was used as with rats. In subsequent passages, the amounts of active virus added to the material of the site of injection were decreased, the interval between the last dose and the time of sacrificing the animals was increased and greater amounts of parts of the central nervous system, other than the site of injection, were used. A summary of the passages is as follows:

1. *First 10 passages.* A mixture of equal amounts of a suspension of brain tissue from the site of inoculation and active virus was given to mice twice daily. The animals were sacrificed for passage 1 to 3 hours after the second dose.

2. *Passage 10 to 15.* Serial passages were carried out every 36 hours. The animals were sacrificed 4 hours after the second dose. Mixtures injected consisted of 2 parts of passage material to 1 part of active virus.

3. *Passage 15 to 20.* Mice were sacrificed 8 to 12 hours after the 3rd injection of a combination of 1 part of active virus to 3 parts of transfer material.

4. *Passage 20 to 25.* Material transferred was made up of 3 parts of brain and brain stem and one part of active virus. The animals received 4 injections over a period of 48 hours and were sacrificed at 12 to 18 hours after the last injection.

5. *Passage 26 to 33.* Mice were sacrificed at 12 to 18 hours after the last injection of a series of 4 given over a period of 3 days.

6. *Passage 33 to 45.* Serial passages were carried out every 4 days, during which time the animals received 4 doses. The animals were sacrificed 24 hours after the fourth dose. The passage material was a mixture of 1 part of active virus and 4 parts of a suspension of mouse brain and brain stem. The material from the 24th and 45th passages injected into a series of mice in multiple inoculations, produced no effect in the mice nor did the virus in either of these passages survive for a longer time than in the preliminary experiment, when it was demonstrated in the mouse brain, 3 but not 5 days after intracerebral injection.

Inasmuch as Nungester[4] reported a paralysis in mice using poliomyelitis virus and mucin, attempts were made to produce the disease by the addition of mucin to the active virus and the material of the 24th passage. Results of this experiment were negative.

In the case of several virus diseases, young animals have been reported to be more susceptible than fully matured animals, so a series of five, 3 weeks old mice and 20 guinea pigs, 14 hours to

[4] Nungester, W. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1128.

2 days old were injected intracerebrally and intraperitoneally, but with negative results.

Attempts to Reduce the Resistance of Animals so as to Render Them More Susceptible. The first method used was to blockade the reticuloendothelial system by the intravenous inoculation of colloid particles. The colloid used was thorium dioxide (thorocotrast), which was kindly supplied by Mr. F. A. Degener of the Heyden Chemical Corporation, New York City. From the literature and from experiments I have carried out with staphylococcal infection in rabbits it appears that the resistance to bacterial diseases can be reduced by doses of colloid particles, large enough to paralyze or partially paralyze the reticuloendothelial system. Using rabbits, 8 cc. per kilogram was injected. However, no attempt was made to determine whether or not the blockade was complete by bilirubin determinations. The dose used gave the usual marked leucocytic blood changes, consisting of a rapid drop followed by a rapid rise in the number of white cells.[5, 6] For mice, almost double the rabbit dose per kilogram of body weight was used, which was very close to the lethal dose. Soon after the intravenous injection of the thorocotrast, the animals received a dose of 20% suspension of active virus intracerebrally and intraperitoneally. In the case of the mice, the 40th serial passage material was also used.

Although little evidence exists for the belief that resistance to poliomyelitis is connected with the glands of internal secretion, an attempt was made to produce the disease in animals in which the hypophysis had been removed, inasmuch as its removal produces atrophy and probably loss of function of all the internal secretory glands. The removal was carried out by Dr. Hans Selye of the Department of Biochemistry, McGill University, Montreal. Single inoculations and a series of 16 passages in animals with the glands removed, were done. Serial transfers were carried out as described above and all with negative results.

Summary. From these experiments, it appears that of all the ordinary laboratory animals, the mouse should be the best in attempting to produce poliomyelitis, for the virus survives in its brain for a longer time than in that of the guinea pig, rabbit or rat. Serial passage of the virus of poliomyelitis, for 45 generations in the mouse and 16 in the rat, failed to adopt the virus to either host. Likewise, the use of very young mice or guinea pigs proved ineffective. Blockade of the reticuloendothelial system with thorium

[5] Elvidge, A. R., *J. Path. and Bact.*, 1928, **31**, 33.

[6] Gottlieb, R., *Can. Med. Assn. J.*, 1933, **28**, 496.

dioxide failed to reduce the resistance to the disease in mice or rabbits, nor did the addition of mucin to the virus aid in using mice, or hypophysectomy in using rats.

7877 P

Cataract Formation in Rats Fed on a Diet Containing Galactose.*

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Mitchell and Dodge[1] report the occurrence of cataracts in rats fed on a high lactose diet. A similar investigation conducted in our laboratory corroborated their ocular findings. It was therefore considered of interest to determine whether cataracts could be provoked with galactose.

Eight female albino rats, 21 days old and weighing approximately 40 gm. were fed a diet consisting of 50% galactose, 20% cornstarch, 15% caseine, 9% crisco, 4% (Osborn and Mendel) salt mixture, and 2% cod liver oil. In addition, 5 drops of cod liver oil and 0.5 gm. dried yeast powder were fed separately each day.

The rats on this diet appeared well nourished and grew as well as the animals on the standard laboratory ration described by Bing and Smith.[2] In the course of 12 to 14 days, the young animals developed changes in the lens of the eye. The manifestation was bilateral. It was apparent from daily examination of the eyes that the lenticular changes developed in the nucleus of the lens.

Six animals were sacrificed for histological study and 2 were allowed to stay on the same diet. At autopsy no gross pathological changes were noted. The liver, kidney, adrenal, thyroid, and parathyroid glands were fixed for future histological study.

At the end of 7 weeks the remaining 2 animals have matured cataracts in both eyes and are still growing well. They show none of the deficiency manifestations noted in albino rats on diets lacking vitamin A or G. At no time during the experiment was a gastrointestinal disturbance noted.

* Aided by a grant from the Research Funds of the Yale University School of Medicine.

[1] Mitchell, H. S., and Dodge, W. M., Jr., *J. Nutrition*, 1935, **9**, 37.

[2] Smith, A. H., and Bing, F. C., *J. Nutrition*, 1928, **1**, 129.

Because of the nuclear changes in the lens of young animals it was important to determine the effect of a similar diet on older animals. Two albino rats, 64 days old and weighing 150 and 157 gm. respectively, and 2 animals 78 days old, weighing 144 and 159 gm. respectively, were placed on the aforementioned galactose diet. They continued to grow and maintain good health. Between the 18th and 23rd day, they developed changes in the lens. Daily examination of the eyes revealed opacities in the periphery of the lens. When allowed to continue on the diet, the nucleus of the lens also became opaque or cataractous. Two of the 4 animals were sacrificed for histological study of the eyes and the internal organs. On gross examination, no pathological changes were noted. A study of the urine output of the animals showed a marked reduction of Benedict's quantitative sugar reagent. It was calculated that 1 cc. of urine showed 52 mg. of galactose.[3]

A new group of animals have been placed on diets of varying amounts of galactose to determine whether the blood chemistry is similar to that found in rats on a high lactose diet. A more detailed histological study of the ocular changes is contemplated.

It is safe to speculate that galactose given in the diet interferes with the metabolism of the lens. The changes take place in the growing areas of the tissue—in the young albino rat the nucleus is altered whereas in the adult rat, the periphery of the lens is involved.

7878 P

Nature of Nitrogenous Constituents in Petroleum Ether Extract of Plasma.

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Neither the amino nor the non-amino nitrogen obtained in the petroleum ether extract of plasma by the technique of Kirk, Page, and Van Slyke[1] derives entirely from phosphatides. The N:P ratio for lecithin and cephalin is 1, while the Amino N:P ratios are 0 and 1 respectively. In a mixture of these phosphatides the N:P ratio would accordingly be 1, and the Amino N:P ratio between 0 and 1, according to the proportion of cephalin present. Petroleum ether

[3] Roe, J. H., and Schwartzman, A. S., *Am. J. M. S.*, 1933, **186**, 455.

[1] Kirk, E., Page, I. H., and Van Slyke, D. D., *J. Biol. Chem.*, 1934, **106**, 203.

extracts from 10 normal plasmas were prepared and analyzed according to the method of Kirk, Page, and Van Slyke. The N:P ratios varied from 1.7 to 5.5, and averaged 3.4. The Amino N:P ratios varied from 0.6 to 1.7, and averaged 1.7. There was accordingly more of both total N and amino N present in the extract than could be combined in these phosphatides. In extracts from plasma of uremic patients the excess of N and NH_2 was still greater. The N:P ratio in 8 cases varied from 3 to 18, and averaged 9, while the Amino N:P ratio ranged from 0.9 to 4.7 and averaged 2.5. Part of both the amino and the non-amino nitrogen is removable from the petroleum ether by shaking with acidified water. The nature of the material is being studied further.

7879 C

Infection of Monkeys with the Virus of Poliomyelitis in Human Spinal Cords.*

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With 4 out of 5 human cords taken from acute cases of poliomyelitis at autopsy the virus was successfully transmitted to *Macacus rhesus* monkeys. All 5 individuals had died of respiratory paralysis in the acute stage of the disease. Histopathological study showed intense infiltration and considerable nerve cell destruction.

The first of these, strain B, was obtained from a child who died of bulbar paralysis, 5 days after the onset of paralysis, during an outbreak in Montreal, in 1931. More than 3 years later, during which interval the cord and tonsils were left in glycerine, at ice box temperature, a single intracerebral and intraperitoneal injection of each tissue was given to a monkey. The cord produced paralysis in 13 days and the animal was prostrate 4 days later, while the tonsil gave complete paralysis within 6 days.

The second strain, 1049, from a case occurring in New York in 1933, and had been in glycerine for a year when tested, was given in combined intracerebral and intraperitoneal inoculations over 3

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consecutive days and produced a paralysis 5 days later. Repeated intracerebral and intraperitoneal injections of liver, spleen, mesenteric and submaxillary glands, and kidneys failed to show the presence of virus.

The third strain, J, from a case who had died of bulbar involvement on the 6th day of progressive paralysis, had been in glycerine a year prior to inoculation. A combined intracerebral and intraperitoneal injection brought on paralysis in 8 days. Of special interest was the rapid rise of infectivity during 3 serial passages in monkeys and then its sudden drop.

In Table I, the M.C.P. (minimal completely paralyzing dose) of this strain during these monkey transmissions is compared with the infectivity of the fixed monkey strain.

TABLE I.

Passage No.	M.C.P. Dose	Result	M.C.P. dose of fixed monkey passage strain in cc. of 5% suspension
2	0.0006	Paralysis 14 days	0.0003
	0.0025	" 6 "	
3	0.0006	" 7 "	0.0006
4	0.0025	" 8 "	0.0025

It is interesting to note that after the first monkey passage the virus had reached an infectivity almost equal to that of the passage strain and that after 2 such passages it was just as infective. The fifth passage virus suspension had only ¼ the infectivity of the monkey passage strain and after 7 months of storage in the ice box, it failed to infect.

The cord of the fourth strain, H., was taken from a bulbar spinal case who died on the second day of paralysis. The spinal cord was preserved in glycerine for 15 months at ice box temperature. In this instance, the infectivity of the human cord was determined and it was found that 0.1 cc. of a 5% suspension gave an incomplete paralysis, whereas 0.2 cc. gave paralysis in 2 monkeys with the onset of paralysis in 7 and 10 days, respectively. The infectivity of the virus during the next 2 passages as shown in Table II did not rise as rapidly as in the previous cord.

No virus was demonstrable in the liver, spleen, mesenteric lymph

TABLE II.

Passage No.	M.C.P. Dose cc. of 5% suspension	Result	M.C.P. dose of passage virus in cc. of 5% suspension
1	0.2	Paralysis 7 days	0.0006
		" 7 "	
2	0.05	" 8 "	0.0006

nodes, or submaxillary glands using as the dose 1 cc. of a 20% suspension given intracerebrally and 5 to 10 cc. injected intraperitoneally. This injection was repeated in 10 to 11 days. On the other hand 1 cc. of a tonsil suspension inoculated intracerebrally and 3 cc. intraperitoneally gave paralysis in 8 days and the animal was prostrate the next day.

The several points of interest brought out in this study are as follows: 1. The virus isolated from the human was still infective after remaining in glycerine for more than 3 years, which observation is in keeping with that of others for the animal-fixed virus. 2. The virus was transferred with relative ease from the human to the monkey and produced a rapid and complete paralysis in a relatively short incubation period in 4 out of 5 animals. With one cord, as little as 0.1 cc. of a 5% suspension gave incomplete and 0.2 cc. gave complete paralysis. 3. The rapid fixation of the virus for the monkey with one cord specimen in which case, after the second serial passage, it became almost as virulent and, after the third, equally as virulent as the passage strain. This same strain was lost in the fifth passage after 7 months of storage on ice, although 3 months previously it was still infective. This is difficult to explain since the human material had been in glycerine for over a year. This sudden loss of infectivity after several passages has been described by others. 4. In 2 instances virus was not found outside of the central nervous system, although it was demonstrated in the cord. Likewise, Landsteiner, *et al.*,[1] and Webster[2] failed to demonstrate virus in the lymph nodes of human cases while Leiner and von Wiesner,[3] Strauss and Huntoon,[4] Flexner and Clark[5] and others failed to find virus in the blood stream. Flexner and Lewis,[6] on the other hand, demonstrated virus in the mesenteric lymph nodes on one occasion. Römer and Joseph[7] and Leiner and von Wiesner[8] found active virus in the mesenteric nodes of intracerebrally inoculated monkeys. They suggested that the virus had reached the glands from the cord. Our own studies give evidence for this belief, for it has been pointed out that the virus is probably in the

[1] Landsteiner, C., Levaditi, A., and Pasteur, *Compt. rend. Acad. de Sc.*, 1911, **152**, 1701.

[2] Webster, R., *Med. J. of Australia*, 1919, **6**, 21.

[3] Leiner, C., and vonWiesner, R., *Wien Clin. Wchnschr.*, 1910, **23**, 91.

[4] Strauss, I., and Huntoon, F. M., *New York Med. J.*, 1910, **91**, 64.

[5] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, **57**, 1685.

[6] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, **54**, 1140.

[7] Römer and Joseph, *Munchen Med. Wchnschr.*, **57**, 1059.

[8] Liener und von Wiesner, *Wien. Clin. Wchnschr.*, 1910, **22**, 1698.

neurones, and combined immunological and histopathological studies carried out after the height of paralysis suggest its removal from the nerve cells to the perivascular spaces by means of phagocytic cells. Thus, the finding of virus outside of the central nervous system in the human on a single occasion does not imply a systemic disease, inasmuch as in the experimental animal, where the disease seems entirely neurotropic[9] the virus may find its way out of the central nervous system. More important are the more frequent negative results obtained in attempts to isolate virus outside the central nervous system of humans, which fits in with the nonspecific inconstant histopathological findings outside the central nervous system. In 7 acute human cases examined, slight changes were found in the lymphoid tissue. The mesenteric lymph nodes, Peyer's patches and tonsils showed slight catarrhal inflammation with swelling of the germinal centres, some necrosis of the reticular cells and disintegration of the lymphoid elements and slight infiltration with large mononuclear, plasma and polymorphonuclear leukocytes. The spleen showed swelling and disintegration of the reticular cells, while the thymus showed thickening and degenerative necrosis of Hassal's corpuscles, especially in the center, and slight infiltration with polylobed cells. These changes are certainly not significant for poliomyelitis infection, for they may be present in acute febrile conditions. These changes were not constant. The results of the histopathological studies together with the inability to isolate virus outside of the central nervous system, suggest the human disease to be entirely neurotropic. 5. It is interesting to note the relative ease with which virus was obtained from the tonsils in the 2 cases examined. Landsteiner, *et al.*,[1] Flexner and Clark[5] and others have isolated the virus from the tonsils and inasmuch as the nasopharynx[7] is probably the portal of entry for the virus, it is quite likely that the virus has entered due to contact with an infective source and has persisted there.

[9] Brodie, M., and Elvidge, A. R., *Science*, 1934, **79**, 235.

7880 C

Pituitary Hormones and the Blood Sugar Level.

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It has been reported from several sources[1, 2, 3] that glycosuria was obtained after the injection of anterior pituitary extract. Houssay[4] reports that daily injections of the extract into dogs cause hyperglycemia, glycosuria and ketonuria. Evans, *et al.*,[5] observed glycosuria in 2 dogs treated with growth hormone. Barnes, Regan, Nelson[6] found that Amniotin injections reduced glycosuria in depancreatized dogs. Houssay and Biasotti[7] injected rats with the Evans and Simpson extract for growth. Five to 7 injections were given, intraperitoneally, once a day in doses of 10 cc. per rat. Glycosuria and glycemia occurred 2 to 3 days after the cessation of the injections.

In view of these researches it was thought worth while to try the effect of the various pituitary hormones upon the blood sugar level of the rat. Growth hormone (Squibb); pregnancy urine extract, Antuitrin S (Parke-Davis); theelin (Parke-Davis) and Antuitrin G (Parke-Davis Growth Hormone) were used. All the determinations were made by the Somogyi Micro Method[8] for blood sugar. The following summaries give the different experiments with their results.

Antuitrin S. Ten rats were injected daily, subcutaneously, with 1 cc. of the extract for 8 days. Blood sugar was determined 1 day and 4 days after the last injection. No change in the blood sugar level was found. With 4 other rats 2 were injected subcutaneously and 2 intraperitoneally with ¼ cc. twice a day for 4 days. On the 5th day the blood sugars were normal.

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[1] Barnes, B. O., and Regan, J. F., *Endocrinol.*, 1933, **17**, 522.

[2] Baumann and Marine, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1220.

[3] Houssay, Biasotti and Rietti, *Rev. Soc. Argent. Biol.*, 1932, **8**, 469. (Cited by Barnes and Regan, *Endocrinol.*, 1933, **17**, 522.)

[4] Houssay, B. A., *Klin. Woch.*, 1933, **12**, 773.

[5] Evans, Meyer, Simpson and Reichert, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 857.

[6] Barnes, B. O., Regan, J. F., and Nelson, W. O., *J. Am. Med. Assn.*, 1933, **101**, 926.

[7] Houssay, Biasotti and Rietti, *Compt. Rend. Soc. Biol.*, 1932, **111**, 479.

[8] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

Antuitrin G. Six rats were injected subcutaneously with ¼ cc. per day. Blood sugars determined on the 3rd and 8th days were normal. The dose was then stepped up to ½ cc. Two days later the blood sugars were normal. Injections were continued but by the intraperitoneal route for 3 more days after which time the blood sugar level was still normal.

Theelin. Five rats were injected subcutaneously with ¼ cc. per day. The blood sugars were determined on the 3rd, 4th and 8th days. All were normal. The injections were continued, but with ½ cc. per day. Blood sugars determined on the 10th and 13th days were normal.

Growth Hormone, Squibb. Ten rats were given 1 cc. daily, subcutaneously, for 9 days. Blood sugars were determined on the 3rd and 4th days after the injections were discontinued. All were normal. Five rats injected subcutaneously, daily, with ¼ cc. showed no change in the blood sugar level on the 3rd and 8th days. On the 8th day the dose was increased to ½ cc. daily, but no changes were observed 2 and 5 days later.

The totally negative results were surprising. We endeavored in every case to give doses within the physiological range. In one case we deviated from the dosage used. Houssay and Biasotti[7] injected 10 cc. intraperitoneally into 200 gm. rats, a volume which we considered sufficient to distend the abdomen of the animal.

We wish to express our sincerest gratitude to Dr. P. E. Smith of the Department of Anatomy for his kindly suggestions and help during this research. We also extend our thanks to Dr. J. A. Morrell of E. R. Squibb and Son, and to Dr. Oliver Kamm of Parke-Davis for the hormones used in this work.

7881 C

Effect of Hypothyroidism on Antidiuretic Action of Pressor Principle of Posterior Pituitary.

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The effects of total ablation of the normal thyroid gland in man on the action of injected adrenalin and of insulin have been reported

previously.[1, 2] Studies of the effect of total thyroidectomy on the action of injected pressor principle of the posterior lobe of the pituitary are presented below. The antidiuretic effect, and blood pressure and heart rate following the subcutaneous injection of pitressin* have been studied. Several observations support the idea that the thyroid gland is a factor in maintaining water balance. Whether the antidiuretic action of pitressin is altered after thyroidectomy has not been established.

We have compared the effect of pitressin in postoperative hypothyroidism with its effect in subjects with normal thyroid function. Studies were made in 3 subjects with normal thyroid function, in 3 subjects with clinical and laboratory evidence of hypothyroidism which developed after total thyroidectomy, and in 1 additional subject both before operation and subsequently when hypothyroidism had developed. The elapsed time between operation and the pitressin studies varied from 1 to 9 months. Thyroidectomy in 2 of the 4 patients operated was performed for the relief of congestive heart failure, in the other 2 for angina pectoris, in accord with considerations outlined elsewhere by Blumgart and his associates.[3, 4] The basal metabolic rates of the 4 hypothyroid patients studied ranged between —19 and —36% and averaged —27%, clinical signs varying from those of mild hypothyroidism to rather marked myxedema were present. The antidiuretic effect of pitressin in each instance was evaluated by studying the urine output after a liter of water, both on a control day and on a day when pitressin was administered.[5]

The following regimen of study was utilized: at 7:00 A. M., the patient received a standard light breakfast with no coffee or added salt. At 9:00 A. M., with the patient in the recumbent position, blood pressure and heart rate measurements were made at 5-minute intervals until constant readings were obtained. The patient then emptied his bladder, and either 0.10 or 0.15 cc. of pitressin was administered subcutaneously, the site of injection being massaged. (On the control day pitressin was omitted.) The patient then in-

[1] Abrams, M. I., and Gilligan, D. Rourke, *Am. J. Med. Sci.*, 1934, **188**, 796.

[2] Riseman, J. E. F., Gilligan, D. Rourke, and Blumgart, H. L., *Arch. Int. Med.*, in press.

* Pitressin (Parke, Davis Co.) contains 20 pressor units per cc.

[3] Blumgart, H. L., Levine, S. A., and Berlin, D. D., *Arch. Int. Med.*, 1933, **51**, 866.

[4] Blumgart, H. L., Berlin, D. D., Davis, D., Riseman, J. E. F., and Weinstein, A. A., *J. Am. Med. Assn.*, 1935, **104**, 17.

[5] Gargle, S. L., Gilligan, D. Rourke, and Blumgart, H. L., *N. Eng. J. Med.*, 1928, **198**, 169.

gested 1000 cc. of water. The urine voided between 9 A. M. and 2:00 P. M. was collected at hourly intervals: the volume, specific gravity, and chloride content of the 5 specimens were measured. The chloride contents of the urine were measured by the method of Folin.[6] Blood pressure and heart rate measurements were made every 15 minutes for the first hour following pitressin injection, and at 1½ and 2 hours after injection. The results were compared with those obtained on a control day. Basal metabolic rate measurements were made with a Benedict-Roth apparatus, the results being calculated with reference to the Aub-DuBois normal standards.[7]

The diuresis following a liter of water on control days was similar in the patients with normal thyroid function and those with hypothyroidism.

The subcutaneous injection of 0.15 cc. of pitressin caused a marked delay in the excretion of the ingested water in 3 subjects with normal thyroid activity. When no pitressin was given the total 5-hour urine volume was approximately 1000 cc., whereas after pitressin, the volume averaged 350 cc. The most marked antidiuretic effect was evident during the first 2 hours after pitressin when

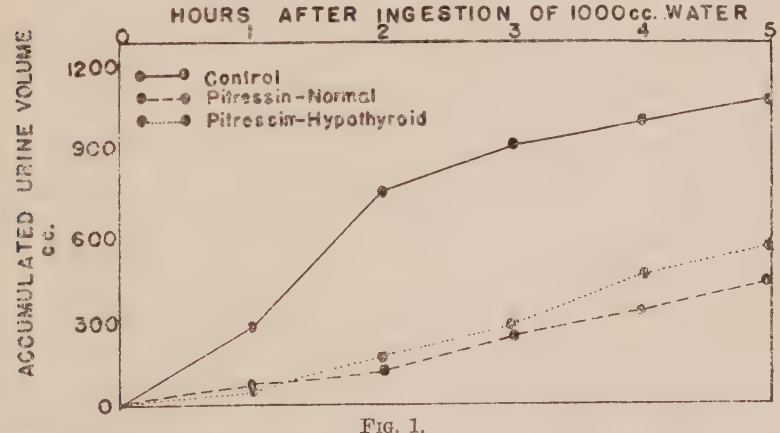

FIG. 1.

Average Antidiuretic Effect of Pitressin in Hypothyroid and Normal Subjects. The urine volumes at a given hour represent the total amounts excreted up to that hour. The "Control" curve represents average of 8 experiments without pitressin. "Pitressin-Hypothyroid" and "Pitressin-Normal" represent average of 4 studies each after pitressin in hypothyroid and normal individuals. Results obtained after 0.10 and 0.15 cc. pitressin have been averaged together since the difference in effect was small.

[6] Folin, O., Laboratory Manual of Biological Chemistry, 1926, Ed. 4, 167. D. Appleton and Co., New York.

[7] Aub, J. C., and DuBois, E. F., *Arch. Int. Med.*, 1917, **19**, 823.

the urine volume averaged approximately 100 cc., as compared with 750 cc. on control days. The antidiuretic effect of 0.10 cc. of pitressin in one additional subject with normal thyroid activity was slightly less prolonged than in the subjects who received 0.15 cc. of this drug. The administration of pitressin always caused an increased specific gravity of the urine and usually caused a decreased chloride output.[5]

In 4 patients with hypothyroidism 0.10 to 0.15 cc. of pitressin likewise caused a decreased urinary volume, an increased specific gravity, and a decreased chloride output; the degree of antidiuretic effect in these patients was not significantly different from that obtained in the subjects with normal thyroid activity (Fig. 1). Comparison of the results obtained in one patient before and after total thyroidectomy revealed no change in sensitivity to pitressin.

The heart rate and blood pressure were not significantly affected in any of the subjects by injections of 0.10 or 0.15 cc. of pitressin. After the intramuscular injection of larger doses of pitressin (0.4 cc.) in normal individuals Grollman and Geiling[8] observed consistently a slight blood pressure rise.

In one subject 0.10 cc. of pitressin, and in another 0.15 cc. caused mild abdominal cramps, nausea, and pallor persisting for about one hour after injection. In a third patient, receiving 0.15 cc. pitressin, the study was terminated because of marked discomfort from these symptoms. One of these subjects who had subjective effects after pitressin was normal; the other 2 were hypothyroid.

We have found no previous reports of the effect of thyroidectomy on the action of posterior pituitary extracts. Clark[9] and Appel,[10] have reported that dogs fed with large doses of whole thyroid died of cardiac depression within a few minutes after 0.2 cc. of pitressin intravenously.

Conclusions. The antidiuretic effect of the pressor principle of posterior lobe pituitary (pitressin) injected subcutaneously is the same in patients with hypothyroidism as in subjects with normal thyroid function. The blood pressure and heart rate are not appreciably affected, either in normal subjects or in patients with hypothyroidism, by the subcutaneous injections of 0.10 and 0.15 of pitressin.

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[8] Grollman, A., and Geiling, E. M. K., *J. Pharm. and Exp. Therap.*, 1932, **46**, 447.

[9] Clark, A. G., *J. Physiol.*, 1929, **68**, 166.

[10] Appel, S., *Arch. f. Exp. Path. u. Pharm.*, 1932, **168**, 726.

7882 C

Comparatively Low Levels of Oestrin in Cases of Chorioepithelioma and Hydatidiform Mole.

GEORGE VAN S. SMITH AND O. WATKINS SMITH. (Introduced by R. N. Nye.)

From the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass.

Although there have been numerous reports of the findings of tremendous amounts of the anterior-pituitary-like hormone (A.P.L.) in the blood and urine of cases of hydatidiform mole and chorioepithelioma, we have found no mention of determinations of oestrin in these patients, and it has even been assumed[1] that the oestrin is also very high. This assumption is presumably based upon the fact that in pregnancy both of these hormones are easily demonstrable.

In the course of our quantitative studies of A.P.L. and oestrin in pregnancy, we have had access to the blood and urine of 4 cases of chorioepithelioma, 3 of them males. The same methods of analysis as those previously described[2] have been used. The results in Table I demonstrate that although the blood and urine of these patients contain a higher concentration of A.P.L. than is ever found in normal pregnancy, the oestrin content is very low, in fact not demonstrable without concentration of the specimens by extraction. In cases 3 and 4, 700 cc. of urine were extracted according to the method of Kurzrok.[3] The amount of oestrin excreted in 24 hours is somewhat more than that of the normal men whose urines we have extracted by the same method, about the same as that of a case of testicular embryoma, but by no means comparable with the amount usually found even in very early pregnancy.[4]

Case 1, a woman who was admitted to the Free Hospital for Women dying of chorioepithelioma, received intravenously 27 cc. of a special preparation of Theelin (kindly supplied by Parke, Davis and Company) containing 1000 r.u. per cc. A drop in the A.P.L. level in both blood and urine followed. Oestrin still was not demonstrable in 5 cc. of serum, however, and the urinary excretion

[1] Geschickter, C. F., Lewis, D., and Hartman, C. G., *Am. J. Cancer*, 1934, **21**, 828.

[2] Smith, G. V., and Smith, O. W., *Am. J. Physiol.*, 1934, **107**, 128.

[3] Kurzrok, R., and Ratner, S., *Am. J. Obst. and Gyn.*, 1932, **23**, 689.

[4] Smith, G. V., and Smith, O. W., to be published in *Surg., Gyn. and Obst.*, 1935.

TABLE I.
Quantitative analyses of A.P.L. and oestrin in blood and urine of cases of chorioepithelioma.

Case	Clinical record	A.P.L		Oestrin	
		Serum r.u. per 100 cc.	Urine r.u. per 24°	Serum	Urine r.u. per 24°
1 Female	Chorioepithelioma with metastases				
	7/11/33	10,000	14,000	Negative with 5 cc.	Less than 100
	7/21/33	10,000	15,000	Negative with 5 cc.	Less than 64
	7/21-24/33—27000 r.u. Theelin i.v.				
	7/24/33—1 d. after Theelin	3,300	8,200	Negative with 5 cc.	95
	7/28/33—4 d. after Theelin Died in P. M.		10,500		105
2 Male	4/13/32—Had embryoma of testis and a lymph gland which had chorioepithelioma surgically removed. Since then has been receiving X-ray Blood and urine 7/13/33	10,000	3,700	Negative with 5 cc.	Less than 100
3 Male	Chorioepithelioma of testis	10,000	9,200	Negative with 10 cc.	26
4 Male	" " "	2,000	7,500	Negative with 10 cc.	21

TABLE II.
Quantitative analyses of A.P.L. and oestrin in one hydatidiform mole compared with those in the placentas from 4 interrupted pregnancies.

Materials analyzed	A.P.L r.u. per gm. of dried powder		Oestrin r.u. per gm. of dried powder	
Hydatidiform mole Began flowing at 3 months Mole passed at 6 months	Sample 1 120	Sample 2 180	Sample 1 0	Sample 2 0
	Maternal portion	Fetal portion	Maternal portion	Fetal portion
Placenta from hysterectomy at 2½ months	12	24	0	4
Placenta from hysterectomy at 4 months	18	30	0	8
Placenta from induced miscarriage at 5 months	9	3	10	10
Placenta from spontaneous miscarriage at 6 months	20	20	10	10

after injections was very much less than would be expected. Similar results followed the oral administration of 24,000 r.u. of Progynon to a woman 8 months pregnant and with the high A.P.L. of late

pregnancy toxemia.[2] (Case 21.) Conversely, a drop in the level of oestrin has been demonstrated following the administration of A.P.L. to cases of threatened miscarriage.[5] These findings might be interpreted as indicating a mutually antagonistic reaction between A.P.L. and oestrin, and the lack of oestrin in cases of chorioepithelioma may be due to the inhibition or destruction of this hormone by tremendous amounts of A.P.L.

In Table II the A.P.L. and oestrin contents of 4 placentas from pregnancies interrupted between the 2nd and 6th months are compared with those of a hydatidiform mole. The same methods of extraction were employed as those described in the analyses of term placentas.[6] There was a marked excess of A.P.L. in the mole but oestrin was not demonstrable.

These few results do not prove that oestrin may never be present in larger amounts in the blood and urine of cases of chorioepithelioma and mole. In female cases in which there are associated luteal cysts that may contain as high as 10,000 m.u. of oestrin per liter of fluid,[7] one would certainly expect increased amounts in the blood and urine, but even then the tumor would not be the source. At autopsy our Case 1 did not have cystic ovaries. The data presented indicate that the chorionic cells themselves, when they become neoplastic, do not contain oestrin in amounts comparable with those in the normal placenta.

7883 P

An Analysis of Rate and Amplitude of Breathing.

ROBERT GESELL AND CARL MOYER.

From the Department of Physiology, University of Michigan, Ann Arbor.

Gaseous mixtures of low oxygen content following double vagotomy in the dog produced an augmentation of tidal air with little or no change in rate of breathing. After carotid gland denervation and section of the pulmonary branches of the vagus nerve, which leaves the chemically sensitive aortic nerve endings intact, the effects of anoxemia are similar, but not as great as those following double

[5] Smith, G. V., and Smith, O. W., *J. Am. Med. Assn.*, 1931, **97**, 1857.

[6] Smith, G. V., and Smith, O. W., to be published in *Surg., Gyn. and Obst.*, 1935.

[7] Siegmund, H., *Wien. klin. Wchnschr.*, 1931, **44**, 1045.

cervical vagotomy. With the vagus nerves intact and the carotid glands denervated, anoxemia augmented respiratory rhythm with little or no change in tidal air. Frequently the amplitude of breathing diminished. (See Figs.)

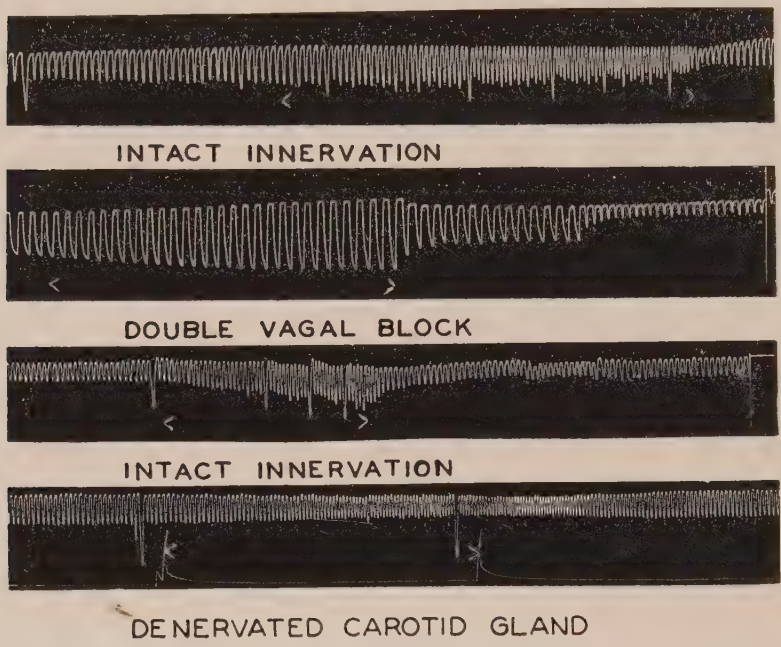

FIG. 1.

In the first and third records, administration of a 4% oxygen mixture to a dog with intact innervation produced the usual results of increased rate and amplitude of breathing. In the second record, during double vagal block and intact carotid gland innervation, similar gaseous administration produced primarily an increased amplitude of breathing. The final acceleration and decreased amplitude is due to deblocking of the vagi during the period of recovery when the animal is breathing room air. In the last record after denervation of the carotid gland, anoxemia produced an increased rate accompanied by a decrease in amplitude. The periods of low oxygen administration extend between the arrow points.

Such results indicate that peripheral chemical stimulation at the carotid gland and at the endings of the aortic nerve contribute importantly to the control of depth of breathing, and that the vagus nerves contribute importantly to the control of rate of breathing. While a differentiation of rate and depth of breathing appears to have been demonstrated, it is not suggested that rate and amplitude control occur exclusively through these 3 channels of nervous influence, for breathing can be accelerated after double cervical vagotomy, and amplitude can be augmented by stimulation of cutaneous sensory nerves.

In some individuals anoxemia produced a greater change in rate of breathing than in amplitude. In others the reverse occurred. In those dogs in which the rate control was developed at the expense of amplitude control, anoxemia produced the same increase in rate after denervation of the carotid gland, and the same small change in amplitude after double vagotomy with carotid gland innervation intact. In those dogs, in which amplitude control was developed at the expense of rate control, anoxemia produced the same large increase in depth of breathing after double vagotomy, and the same small increase in rate after carotid gland denervation with the vagus nerves intact.

The response to carbon dioxide was relatively little affected by denervation of the carotid gland, by double vagotomy, or section of the pulmonary branches of the vagus nerve. The relative absence of increase in rate and the predominance of increase in amplitude from the administration of carbon dioxide with complete innervation may be due to the depressing effect of carbon dioxide on reflexes. Carbon dioxide saturation, so to speak, produces a partial chemical vagotomy, thereby reducing the rate control of the vagus nerves.

7884 C

Immunologic Studies of Anti-Gonadotropic Sera.

CARL BACHMAN. (Introduced by J. B. Collip.)

From the Department of Biochemistry, McGill University, Montreal, Canada.

We have reported[1, 2] that the serum of rats and rabbits treated with anterior-pituitary-like hormone (A.P.L.) of pregnancy urine contained substances capable of protecting test animals against the gonadotropic action of this hormone. The A.P.L. used in these experiments was prepared by alcohol-salt precipitation methods and consequently contained nitrogenous substances giving some of the reactions common to antigenic proteins. It was of interest, therefore, to determine whether the A.P.L.-inhibitory sera of treated animals contained antibodies against A.P.L. in the immunological sense.

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1 Selye, H., Bachman, C., Thomson, D. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1113.

2 Bachman, C., Collip, J. B., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 544.

For this purpose 4 preparations of A.P.L. from different collections of pregnancy urine and one control product prepared by identical methods from male urine were examined. The gonadotropic potency of the pregnancy urine extracts varied between 10 and 100 rat-day-units (R.D.U.) per milligram of dry substance, the differences being accounted for principally by variations in salt content of the preparations. The male urine extract possessed no gonadotropic properties, but like the pregnancy urine extracts, gave the biuret and Millon reactions.

Each of the above preparations was injected intravenously into 5 female rabbits. The A.P.L. treated rabbits received 5 daily increasing doses of from 100 to 1000 R.D.U. in salt solution, and following a rest period of 5 days, a second course of from 500 to 2500 R.D.U. The rabbits treated with male urine extract received similar courses of injections in doses ranging from 10 to 150 mg. of dry substance dissolved in salt solution. The animals were bled from the fourth to the tenth day following the last injection. As previously reported, the serum of rabbits treated with A.P.L. in this manner was highly potent in A.P.L.-inhibitory substance.

When the sera of the treated rabbits were tested for precipitins against homologous injection preparations, preliminary experiments with the "layer" method showed weakly positive results in low dilutions of many of the antigens. This method was therefore abandoned for the more sensitive technic of complement fixation.

In the complement fixation tests 0.2 cc. of heated serum from the treated rabbits was incubated at 37°C. with 1.0 cc. of a 1:20 dilution of fresh pooled guinea pig serum as complement, and 0.2 cc. of urine extract "antigen" in varying dilutions starting from a concentration of 5 mg. (50 to 500 R.D.U.-A.P.L.) per cc. of salt solution. To this mixture, made up to 3.0 cc. with salt solution, was added after one hour 1.0 cc. of a 1:40 dilution of washed sheep corpuscles and 2 units of hemolytic amboceptor. In the proportions of reagents chosen following numerous trials, and in the fact that complement was titrated only after preliminary incubation for one hour, the test set-up provided a sensitive indicator for the presence of complement fixing antibodies. Controls of each dilution of antigen, as well as the usual corpuscle, hemolysin and serum controls were run. In addition to these a control of each serum in the absence of hemolytic amboceptor was also run, in order to reveal the hemolytic activity of serum alone.

It was soon apparent that while many sera from the injected rabbits were strongly hemolytic in themselves, one or 2 from each

group gave satisfactorily graded inhibition of hemolysis in the presence of urine extract. Complement was never bound, however, in higher than 1 :100 dilutions of antigen. Inasmuch as normal rabbit serum controls frequently gave reactions up to 1 in 10 dilution of antigen, it will be noted that the complement fixing titer of potent A.P.L.-inhibitory sera was low.

In one series of rabbits an attempt was made to raise this titer by repeating the A.P.L. injections after a 4 months' rest period. This was not possible. It was found that just before beginning the new injections the original complement fixing titer had been retained both in the preserved serum and *in vivo,* while the A.P.L.-inhibiting potency had been maintained in the preserved serum but was absent in blood drawn at this time. After the new injections the A.P.L.-inhibiting property was restored but complement binding titer was not raised appreciably above the previous level.

When the cross-reactions of the sera were examined, it was found that the sera from the A.P.L.-injected rabbits fixed complement about equally with all A.P.L. antigens, irrespective of the preparation used for treatment. Uniform cross-reactions for comparable dilutions of antigen were, moreover, observed between A.P.L. on the one hand and the sera of the rabbits treated with male urine extract on the other and *vice versa.* Three of the 4 A.P.L.-treated rabbit groups gave cross reactions against human serum in dilutions up to 1 in 5000 to 1 in 25,000. The group treated with the fourth A.P.L. preparation, and the animals injected with male urine extract gave no reactions with human serum. Where this reaction was observed, it was equally strong for normal male and female serum, as well as for pregnancy serum. The reverse of this cross reaction was examined by immunizing 2 series of 5 rabbits each with male serum in the manner above outlined, and testing these sera against urine extract antigens. This procedure, however, gave no consistent cross reactions, although the sera gave titers above 1 in 5,000 against the human serum antigens used for immunization. (Table I.)

A point of difference between the A.P.L.-inhibiting properties and the complement fixing factors of sera has been noted above in the disproportionately rapid loss of inhibitory titer *in vivo* after the injections were discontinued. That these two phenomena do not parallel each other was further suggested by finding that A.P.L.-inhibitory sera from treated male and female rats gave no complement fixation whatever with A.P.L. antigens.

The problem was examined finally as follows: The pooled serum

TABLE I.
Showing maximum dilutions of antigen at which complement fixation was observed.

Antigen	Serum of Rabbits Treated with: A.P.L. No. 1	A.P.L. No. 2	A.P.L. No. 3	Male urine Extract	Human Serum	Normal Rabbit Serum
A.P.L. No. 1	100+	10+	10+	—	—	1+
A.P.L. No. 2	10+	100—	10+	10+	1+	1+
A.P.L No. 3	0	10+	100—	10—	0	1+
Male urine extract	10—	100+	100+	100+	0	1+
Human serum	0	5000	25000	0	5000	1

of rabbits treated with male urine extract, and that of both groups immunized against human serum were assayed for A.P.L.-inhibiting substances by both the rat and rabbit technics described in our previous communications. In no instance was inhibitory activity observed.

We conclude that A.P.L.-treated rabbits yield sera which give weak immunity reactions *in vitro* with solutions of A.P.L and extracts of male urine, and stronger but less constant reactions with human serum proteins. At the present time, however, there appears to be no clear connection between these phenomena and the specific A.P.L.-inhibitory property demonstrable in such sera with biological methods.

7885 C

Cytological Responses of Rat Thyroid to Treatment with Anterior Pituitary and Potassium Iodide.

ANNE COUCH HALPERN. (Introduced by Ivan E. Wallin.)

From the Department of Anatomy, School of Medicine, University of Colorado, Denver.

Intensified mitotic proliferation and an increase of colloid in the thyroid gland of the normal guinea pig, following potassium iodide administration, have been reported by Loeb.[1] As colloid increased in the follicles, he noted that the follicular epithelium became distended and flattened.

Loeb and Bassett[2] reported that dried and powdered anterior pit-

[1] Loeb, Leo, *Am. J. Path.*, 1926, **2**, 19.

[2] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 490.

uitary substance produced an intense thyroid hyperplasia, and stimulated mitosis in the cells of the guinea pig thyroid.

This apparently successful use of potassium iodide and anterior pituitary extracts in producing experimental modifications in thyroid activity suggested the possibility that similar methods might be applied to a study of the behavior of mitochondria and the Golgi apparatus.

Thirty-five healthy, albino rats of both sexes, ranging in age from 75 to 90 days, were used in this study. Sixteen received daily injections of 0.6 cc. of pituitary extract, per animal. This series was divided into 4 groups, as follows: Group I received injections for 3 days; Group II for 5 days; Group III for 7 days, and Group IV for 9 days. The pituitary extract used was prepared according to the method of Loeb and Bassett.[2] Four animals received injections of 0.05 gm. each, of potassium iodide in distilled water, daily for 7 days. All injections were made intraperitoneally. The animals were sacrificed 24 hours after the last injection, and the thyroids removed and fixed immediately. Twelve animals were sacrificed as controls, in the course of the study.

The general histological findings in all groups confirm the work of Loeb and Bassett, although the changes induced by the experimental procedure are less intense than those described by these workers.

In the control glands the mitochondria are in the form of minute and very delicate rods, abundantly scattered throughout the cytoplasm.

In the glands of animals which received potassium iodide injections the mitochondria show a marked decrease in number, and change in form. In addition to the rod-like forms seen in the controls, many of the mitochondria appear as coarse granules, of irregular size. The number of these granular forms in a given cell varies inversely with their size. While the rod-like forms are scattered evenly through the cytoplasm of the entire cell, the granules tend to segregate in the pole of the cell adjacent to the lumen of the alveolus.

Following pituitary treatment there is likewise a change of some of the rod-like mitochondria into granules. It is to be noted, however, that the granules which appear after pituitary treatment are small and of uniform size, in contrast to the coarse, irregular granules which appear following injections of potassium iodide.

In the glands of animals which received pituitary injections for 3 days, the rods and granules are about equal in number. Following 5 days of treatment, the granules are more numerous than the

rods. Their distribution in the cell is somewhat uneven, the greatest number being seen in the portion of the cell bordering the lumen of the alveolus. After 7 to 9 days' treatment, some of the mitochondria appear as filaments, in the form of a lacy network around the nucleus. The bodies and ends of these filaments show blebs, or swellings. It was impossible to determine whether the filaments were continuous strands, or merely lines of adjacent granules. Scattered granular mitochondria are also abundant in these cells, but no rod-like forms can be seen.

The Golgi material in the controls is mainly in the form of irregular particles, scattered throughout the cytoplasm, although it appeared as a reticulum in a few cells.

Following potassium iodide injections, the Golgi material shows no conspicuous variations from the controls.

In the glands of animals which received pituitary treatment from 3 to 7 days there is a marked decrease in the amount of Golgi material present within the follicle cells. Simultaneously Golgi material may be seen in the intercellular regions. After 9 days of treatment the follicle cells show a slight increase in Golgi material, as compared with the animals treated for a shorter period, but this increase is not sufficient to raise the Golgi content of the cells to the control level. Coincident with the increase in Golgi material within the cells, there is a proportionate decrease in the Golgi material in the intercellular regions. Three of the animals which received pituitary treatment did not show any response, but as these animals belonged to different groups, their failure to react may be attributed to individual variation.

The findings on the response of the mitochondria to potassium iodide injections confirm those of Nicholson[3] and Seecoff.[4]

The uniform response of the mitochondria in all the animals which received pituitary injections, suggests that they are more sensitive to changes in cell activity than the Golgi material, which did not respond so consistently.

The fragmented appearance of the Golgi material in the controls is not in agreement with the findings of Ludford and Cramer,[5] who described it as a reticulum in the normal mouse thyroid. In a subsequent paper[6] on the rat gland, they seem to infer that the appear-

[3] Nicholson, F. M., *J. Exp. Med.*, 1924, **39**, 63.

[4] Seecoff, D. P., *Am. J. Path.*, 1925, **1**, 295.

[5] Ludford, R. J., and Cramer, W., *J. Phys.*, 1926, **61**, 398.

[6] Ludford, R. J., and Cramer, W., *Proc. of Roy. Soc., of London* (B), 1928, **104**, 28.

ance is the same in the normal rat gland, although they do not definitely state that they ran controls on the rat studies. They believed that fragmentation of the Golgi apparatus was indicative of hypersecretion. The present study makes it difficult to accept this interpretation since in the controls, neither the appearance of the epithelium, nor the condition of the mitochondria in the cells, presented the characteristics usually associated with intensified activity.

Efforts were made to determine a possible cause of the fragmented appearance of the Golgi apparatus in the controls. The maintenance conditions and the technique employed in preservation and staining the material were carefully checked and additional animals, from different sources, were sacrificed. In all cases the Golgi material presented essentially the same appearance as that seen in the original controls.

7886 C

A Microörganism which Decomposes the Specific Carbohydrate of Pneumococcus Type VIII.

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From samples of uncultivated soil obtained in several localities, a microörganism has been isolated in pure culture, by methods previously described,[1] which decomposes the specific carbohydrate of pneumococcus type VIII. Although marked cross precipitation is obtained with pneumococcus type-VIII specific carbohydrate in type-III antiserum[2] and, conversely, with type-III carbohydrate in type-VIII antiserum, strains of soil bacteria *(B. palustris)*[1] which decompose the carbohydrate of pneumococcus type III do not act on that of type VIII.

The two microörganisms correspond closely in morphology, cultural characters, and in the production of a soluble enzyme; and the new culture should also be classified as *B. palustris*. The vegetative cells are Gram-negative motile rods with peritrichal flagella, usually 6 in number. They vary in width from 0.6 to 0.8μ and in length from 2.5 to 3μ. Oval spores wider than the vegetative cells are

[1] Sickles, G. M., and Shaw, Myrtle, *J. Bact.*, 1934, **28**, 415.

[2] Brown, Rachel, to be published.

formed. Colonies on blood agar are from 1 to 2 mm. in diameter, smooth, moist with somewhat raised crenated edges. The colonies are of 2 types—one, white and opaque; the other, gray and semi-translucent.

Other cultural characters are also very similar to those displayed by the microörganism which utilizes the carbohydrate of pneumococcus type III. Growth was obtained in mineral medium containing 1% of dextrose, saccharose, maltose, dextrin, salicin, xylose, and galactose, but change in the reaction of the medium was negligible. No growth was present in mineral medium containing lactose, inulin, and mannite. The optimal temperature for growth appeared to be about 29°C., but it took place up to 40°C. Maximum growth and enzyme action were obtained at from pH 7.0 to 7.5, although both were present over a wide range. It was possible to concentrate the soluble enzyme by ultrafiltration through a 9½% acetic-acid nitrocellulose membrane.[3]

The criterion for destruction of the carbohydrate was absence of precipitation with the specific immune serum. No activity of the microörganism on the specific carbohydrates of pneumococcus type I, II, or III could be demonstrated. The cross reaction of the type-VIII carbohydrate in type-III antiserum disappeared at the same time as did the specific precipitation with type-VIII serum. The type-VIII carbohydrate, after decomposition by this culture, failed to induce purpura in mice,[2] even in the presence of immune horse serum, which usually has the effect of increasing the purpura.

Mice were protected against 1000 M.F.D. of a fully virulent strain of type VIII by 2 units[4] of the cell-free enzyme and against 10,000 M.F.D. by 4 units. Ten units did not protect mice against 10 M.F.D. of pneumococcus type III.

[3] Quigley, J. J., *Am. J. Hyg.*, 1934, **20**, 218.

[4] Dubos, René, *J. Exp. Med.*, 1932, **55**, 377.

7887 P

The Specific Carbohydrate from Pneumococcus Type VIII.

RACHEL BROWN.

From the Division of Laboratories and Research, New York State Department of Health, Albany.

Following previous work on the specific carbohydrates of the pneumococcus, a study of preparations from a type-VIII strain has been undertaken in order to determine differences in activity and chemical composition. This culture was isolated in 1930 from pneumonic sputum and has since been maintained at a maximum virulence by frequent mouse passage. It is agglutinated by type-III antiserum in low dilution and by type-VIII in high dilution.

Specific carbohydrate was prepared from the cellular sediment and also from the supernatant fluid of from 16- to 20-hour cultures grown in an infusion-free peptone broth[1] which contained 0.2% cane sugar. The cellular extracts were purified by methods similar to those reported for type-I pneumococcus.[2] The soluble specific substance was best removed from the broth concentrates by precipitation as the barium or calcium salt and by repeated alcoholic precipitations. Purification through the calcium salt resulted in the purest product yet obtained, but the yield was low.

The reactions which distinguished the cellular carbohydrate from the soluble specific substance of the type-I pneumococcus failed to indicate any difference in the type-VIII fractions prepared as described. Although both fractions induced purpura, neither was observed to have immunizing activity in mice; furthermore, precipitation tests with adsorbed sera showed no difference between them.

The type-VIII soluble specific substance was readily soluble in water and gave an acid reaction. It passed through collodion membranes rapidly but through cellophane slowly and only after prolonged dialysis. Analysis of one preparation gave 0.19% nitrogen, 0.06% phosphorus, 0.70% ash, and 3.90% moisture. The specific rotation was about +126°. Before hydrolysis there was no reduction of Fehling's solution, but, when boiled for 4 hours with 10% sulfuric acid, the polysaccharide yielded 69.5% of reducing sugars calculated as dextrose. The Molisch test was positive in a 1:2500 dilution and the naphthoresorcinol in a 1:100. In the latter

[1] Wadsworth, Augustus, and Brown, Rachel, *J. Immunol.*, 1931, **21**, 245.

[2] Wadsworth, Augustus, and Brown, Rachel, *J. Immunol.*, 1933, **24**, 349.

concentration, the ninhydrin, biuret, and xanthoproteic-acid tests were negative. The soluble specific substance was readily precipitated by basic and neutral lead acetates, calcium chloride, barium hydroxide, silver nitrate, mercuric and mercurous nitrates but not by copper sulfate, uranium nitrate, ammonium sulfate, tannic acid, picric acid, phosphotungstic acid, nor trichloracetic acid.

The highest dilution of type-VIII soluble specific substance which gave precipitation with type-VIII antipneumococcus serum was 1:4,000,000 and with type-III antiserum 1:2,000,000. The homologous reaction, however, was the stronger. This precipitate tended to be more voluminous and floccular, except in high concentration, while the heterologous precipitate was compact and transparent. When a type-VIII antiserum was adsorbed with type-III soluble specific substance, it still precipitated with the type-VIII carbohydrate in the same dilution as before adsorption; but, when adsorbed with the latter, it failed to precipitate with either. Also, type-III soluble specific substance removed from type-III antipneumococcus serum the precipitins for both types III and VIII soluble specific substance, but type-VIII carbohydrate removed only the homologous precipitins. When tested qualitatively for complement-fixing activity, type-VIII soluble specific substance, in 1:100,000 dilution, reacted with type-VIII antiserum from the rabbit but not with type I, II, or III. The purpura-inducing activity in mice of a preparation of type-VIII cellular carbohydrate was partially neutralized by types III and VIII antipneumococcus sera from the rabbit and was intensified by these two antisera from the horse. Treatment of this carbohydrate solution with pepsin or trypsin did not affect the purpuric activity. The type-VIII specific carbohydrate induced fatal anaphylactic shock in guinea pigs which had been passively sensitized with the homologous antipneumococcus serum from the rabbit but not from the horse.

7888 C

Heterophile Antibodies in Infectious Mononucleosis.

C. A. STUART. (Introduced by A. M. Banta.)

From the Biological Laboratory, Brown University.

It was previously noted[1] that emulsions of guinea pig kidney removed sheep cell agglutinins from the sera of infectious mononucleosis patients slowly and in some cases incompletely even after 3 successive adsorptions with relatively large amounts of tissue. Since ordinarily guinea pig kidney containing heterophile antigen possesses marked affinity for heterophile antibodies it was decided to reinvestigate the antigenic relationship of guinea pig kidney to the sheep cell antibodies occurring in the blood of individuals with infectious mononucleosis.

In the previous work, to conserve material, finely ground emulsions of tissue prepared for immunization purposes were used for adsorption and as the number of successive adsorptions increased with any one serum the turbidity of the supernatant fluid increased considerably. Under these conditions it is possible that the physical properties of the serum, viscosity, surface tension, etc., were so altered that the sheep cells used in the test after adsorption were unable to fix the homologous agglutinins or having fixed the agglutinins were unable to agglutinate in the characteristic manner.

Throughout the work herein reported guinea pig kidney tissue was lightly ground in a mortar, the macerate strained through a coarse fabric and the material passing the cloth washed by centrifugation until the supernatant fluid showed only a slight opalescence. Five infectious mononucleosis sera have been absorbed with such tissue emulsions in the following way: To 2 cc. of a 1:2.5 dilution of the serum were added 0.5 cc. of the tissue emulsion. The tubes were shaken and placed at 37.5°C. for 30 minutes. The tubes were then centrifuged, a portion of the supernatant fluid tested with sheep cells for both agglutinins and lysins (lowest dilution 1:10). Fresh tissue was added to the remainder and the procedure repeated until 3 adsorptions were completed. The sera were adsorbed in the same manner with boiled sheep cells. As a control on the efficiency of the tissue emulsion similar adsorptions and tests were made on the sera of rabbits immunized to guinea pig kidney. The average titer of the different sera before adsorption and after the first, second and third adsorptions will be found in Table I.

[1] Stuart, Burgess, Lawson and Wellman, *Arch. Int. Med.*, 1935, **54**, 199.

TABLE I.

Adsorption of Infectious Mononucleosis Sera and Rabbit Anti-Guinea Pig Kidney Sera with Emulsions of Guinea Pig Kidney.

Infectious Mononucleosis Serum	Before Ads.	After 1st Ads.	After 2nd Ads.	After 3rd Ads.	Rabbit Anti-Guinea Pig Kidney Serum	Before Ads.	After 1st Ads.	After 2nd Ads.	After 3rd Ads.
					Titers for Sheep Cell Agglutinins				
1	1280	1280	640	640	1	160	0	0	0
2	2560	2560	2560	1280	2	160	0	0	0
3	1280	1280	1280	640	3	80	0	0	0
4	5120	2560	2560	2560	4	80	0	0	0
5	640	640	320	320	5	20	0	0	0
Average	2176	1664	1472	1088	Average	100	0	0	0
					Titers for Sheep Cell Lysins				
1	1280	640	640	640	1	40960	0	0	0
2	5120	2560	2560	1280	2	40960	0	0	0
3	2560	2560	1280	1280	3	20480	40	0	0
4	5120	5120	5120	2560	4	20480	40	0	0
5	1280	1280	640	640	5	20480	160	10	0
Average	3072	2432	2048	1280	Average	28672	48	2	0

It is evident from Table I that tissue emulsions prepared in the specified manner while depleting rabbit anti-guinea pig kidney serum of its sheep cell antibodies, frequently in a single adsorption, fails to remove any significant part of these antibodies from infectious mononucleosis sera. Boiled sheep cells, as might be anticipated, adsorb sheep cell antibodies from both the pathological sera and the immune sera.

It is interesting to note that the injection of guinea pig kidney into rabbits produces a high lytic titer for sheep cells but a very low agglutinating titer for the same cells. Prolonged immunization does not alter this condition. On the other hand in infectious mononucleosis sera, the increase in sheep cell agglutinins and lysins are often identical until the maximum point is reached. In several cases, however, we have noted that the lytic titer decreases sooner and more rapidly than the agglutinating titer.

The increased sheep cell antibodies which appear in the blood of infectious mononucleosis patients while heterophile in nature are not of the guinea pig heterophile type.

7889 C

Action of Apomorphine Hydrochloride upon the Small Intestine in Non-anesthetized Dogs.*

CHARLES M. GRUBER AND JOHN T. BRUNDAGE.

From the Department of Pharmacology, Jefferson Medical College, Philadelphia.

Morphine when subjected to a strong acid undergoes a chemical rearrangement becoming apomorphine, an isoquinoline derivative possessing very little narcotic action but a strong emetic action. Cannon[1] investigated the action of apomorphine upon the cat's stomach by the X-ray method. He observed that there was total inhibition of the cardial portion of the organ while there was contraction of the extreme end of the pyloric portion.

As no study of the action of apomorphine upon the intact intestine of the unanesthetized dog seems to have been done this series of experiments was undertaken.

* This research was made possible through a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

1 Cannon, *Am. J. Physiol.*, 1898, **1**, 373.

The method was the same as that employed in another research[2] and will therefore not be described in detail here. The animals were the same as those used in the previous work, 5 having Thiry-Vella loops of the ileum and 3 of the jejunum. They were healthy animals in every respect. Apomorphine, in doses of 0.013 to 0.02 mg. per kg. body weight, dissolved in Ringer's solution, was injected intravenously 10 times in the 5 animals with Thiry-Vella loops of the ileum and 8 times in the 3 animals with jejunal loops.

In every experiment performed on the 3 dogs with Thiry-Vella loops of the ileum and the 4 with similar loops of the jejunum, apomorphine caused a rapid increase in the general tonus which was followed by an abrupt fall during a period of nausea. This was followed by a rather prolonged increase in general tonus. The periods of nausea were evidenced sometimes by vomiting and others simply by profuse salivation and licking reflexes depending on the size of the dose of the drug administered. The inhibition of the jejunum, during the period of nausea, was far more pronounced than that of the ileum. In many experiments, only salivation occurred and yet relaxation of the jejunum was very apparent.

FIG. 1. Female dog weighing 15.5 kg. with a Thiry-Vella loop of the jejunum. In this and the following figure the top curve is that of the intestine and the bottom, the time interval written in 20 seconds. Contraction up stroke, relaxation down stroke in the intestinal record. The balloon had a length of 50 mm. and a diameter of 20 mm. The pressure within it was 15 cm. of water.

At the ↑, 0.3 mg. of apomorphine hydrochloride was injected intravenously. At V, the animal vomited.

Fig. 1 shows a record of the jejunum before and after the injection of apomorphine. At the arrow, 0.3 mg. of apomorphine hydrochloride was injected into an animal weighing 15.5 kg. This was followed by a slight but sudden increase in general tonus which dropped very rapidly to a low level as signs of discomfort, excess salivation and rapid licking to overcome it, became noticeable. At V, the animal vomited and after this the general tonus increased to

[2] Gruber and Brundage, *J. Pharmacol. and Exp. Therap.*, 1935, **53**, 120.

a point above the control level. Subsequently this change was followed by increased peristaltic activity and decreased general tonus.

The ileum responded to the administration of apomorphine less actively than did the jejunum. The loss of tonus of the ileum was always less than that of the jejunum. The writing lever recorded an increase in tonus during each vomiting spell but this is, of course, due to the contraction of the abdominal muscles and is not a direct result of the drug's action. Though the ileum loses some of its tonus during the period of nausea this is far less than that suffered by the jejunum. The effect of increased tonus predominates in the ileum, even small doses producing prolonged increases in general tonus.

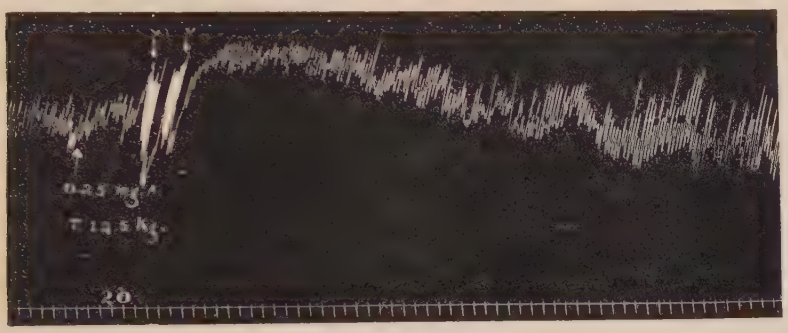

FIG. 2. Female dog weighing 12.5 kg. with a Thiry-Vella loop of the ileum. At the ↑, 0.25 mg. of apomorphine hydrochloride was injected intravenously. At V the animal vomited.

In Fig. 2, an animal weighing 12.5 kg. was given, at the arrow, 0.25 mg. of apomorphine hydrochloride dissolved in Ringer's solution. The general tonus increased shortly after the injection. Within 2 minutes after the injection the animal vomited twice, at V. After cessation of vomiting the tonus recorded a level below that of the control level. A minute later or 3 minutes after the injection the tonus had risen above the control level. A gradual decrease brought it back to normal after 15 minutes.

Conclusions. 1. Apomorphine hydrochloride by causing nausea in the unanesthetized dog, momentarily decreases the general tonus of the ileum and jejunum. 2. Apomorphine, in large enough doses, causes an increase in the general tonus of the ileum and jejunum similar to that produced by morphine. 3. With the use of the jejunal loop it may be determined that apomorphine may cause an increase in the peristaltic activity of the organ and at the same time a decrease in the tonus. This phenomenon is similar to that noted with the use of dilaudid and morphine in these same animals.

7890 C

Failure to Transmit Carcinogenic Agents from the Pregnant Mouse Embryos in utero.

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Several investigators have reported transmission in mice of carcinogenic agents from mother to embryo. One group of such investigators believes that a mash of embryos which have developed in the body of a cancerous mother, can transmit cancer on transplantation in other mice. A diagram of this type of relationship is given in Fig. 1.

It is evident that if a highly specific and effective degree of carcinogenic activity were transmitted in the way described it would be definite proof of the existence of such an agent smaller than and separable from the cell. Since the larger mass of evidence in rodent tumors argues against the probability that such a condition exists, it seemed worth while to make certain experiments along this line.

As far as possible the technique described by Tesauro[1] was followed.

In any mice which showed masses at the site of transplanted embryonic tissue, observations by palpation were made at frequent intervals. The size of masses was indicated by an outline of their approximate area recorded on a chart kept for each animal. In each case histological preparations were made of the masses which persisted. Study of these preparations showed the masses were either teratoid or inflammatory in nature. In no case did a mass resemble the original tumor employed. Similar inflammatory or teratoid growths were obtained in many control animals inoculated with an emulsion of embryo from non-cancerous mothers. The results of the various experiments are given, in a condensed form in Table I. In no case was a malignant tumor obtained in either experimental or control animals, as a result of inoculation with embryonic tissue.

Although negative results are never entirely satisfactory it seemed worth while to record the fact that the results obtained by Tesauro are not universally applicable and that the principle which is involved remains, in so far as our experience is concerned, unestablished.

In this connection it is of interest to note that where a tumor was used, the mice receiving transplants of the embryonic mash were

[1] Tesauro, G., *Z. f. Krebsforsch*, 1931, **35**, 109; *Boll. d. Soc. ital. di biol. spec.*, 1932, **7**, 332; *Arch. di Sc. biol.*, 1932, **17**, 48.

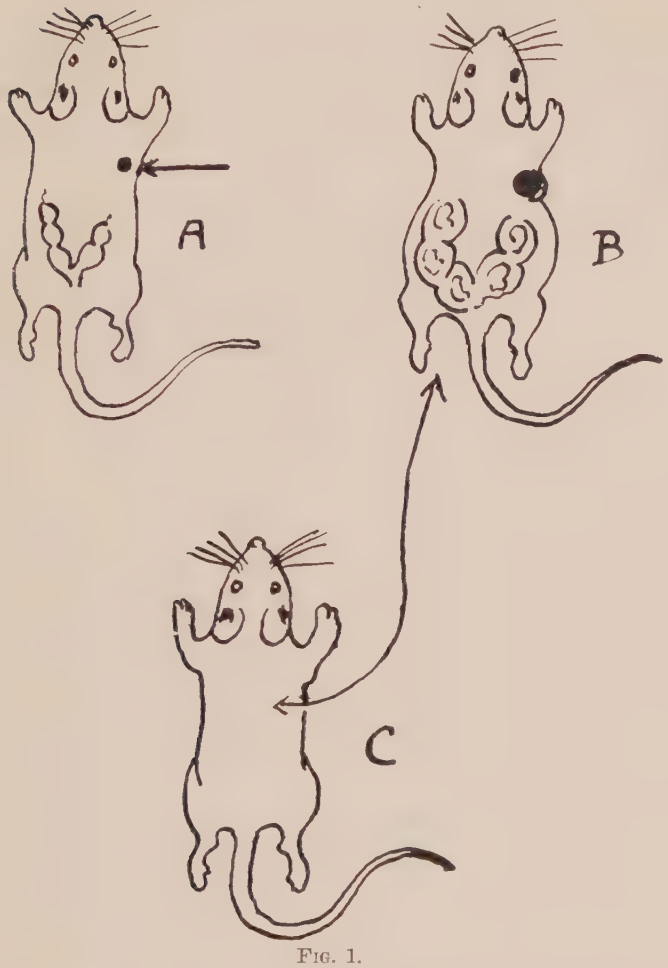

FIG. 1.

A. Early pregnant mouse inoculated with cancer as indicated by arrow.
B. Same mouse later. Tumor has grown. Embryos, with head, tail and limbs removed, are made into emulsion and inoculated into mouse C.
C. Non-pregnant, non-cancerous mouse which receives implant of embryonic emulsion in the site indicated by arrow. This mouse is supposed to grow a tumor of original type (A) at the site of implantation.

of the proper constitution to grow the tumor had the stimulus been provided.

It is also important to point out that sarcoma M 37 was the same tumor used by Tesauro in several of his experiments. It is therefore clear that this tumor does not, under various conditions, behave in the same way as regards the experimental technique under investigation.

TABLE I.

Exper.	Tumor	Pregnant ♀ receiving tumor	Date 1934	Killed and emulsion made	No. receiving crushed embryo	Growing masses	End of experiment	Duration of exper. days
A	Sarc. 15091a	No. 3934	2-16	2-23	1	0	3-25	37
B	,,	No. 4012	2-16	2-24	3	3	3-25	37
C	Carc. F_1 R_4*	No. 3763	2-26	2-26	4	4	3-25	27
D	Carc. d Br B	No. 3933	2-16	2-26	9	9	3-25	37
E	,,	No. 4276	2-16	2-27	6	5	3-25	37
F	Control	No. 3860	3-12	3-12	5	5a	3-25	13
G	,,	No. 4398	3-12	3-12	4	4a	3-25	13
H	Sarc. M37	No. 3877	3-14	3-20	12	3	5-4	51
I	,,	No. 4710	8-27	9-6	6	1	10-11	35
J	,,	No. 5456	8-27	9-6	6	0	10-11	35

* Spontaneous tumor observed in pregnant ♀.

a The masses in the controls were as large or larger than those in the experimental animals and were histologically indistinguishable from them.

Conclusion. In view of the negative results herein reported it would seem that a larger series of tumors with more detailed and more enlarged photographs of the induced tumors should be available before the principle of transference from mother to embryo of a cancer-producing agent can be considered as established.

7891 C

Effect of Poliomyelitis Virus in Baby Monkeys Previously Given Paratyphoid Colon Filtrate and Vaccine.*

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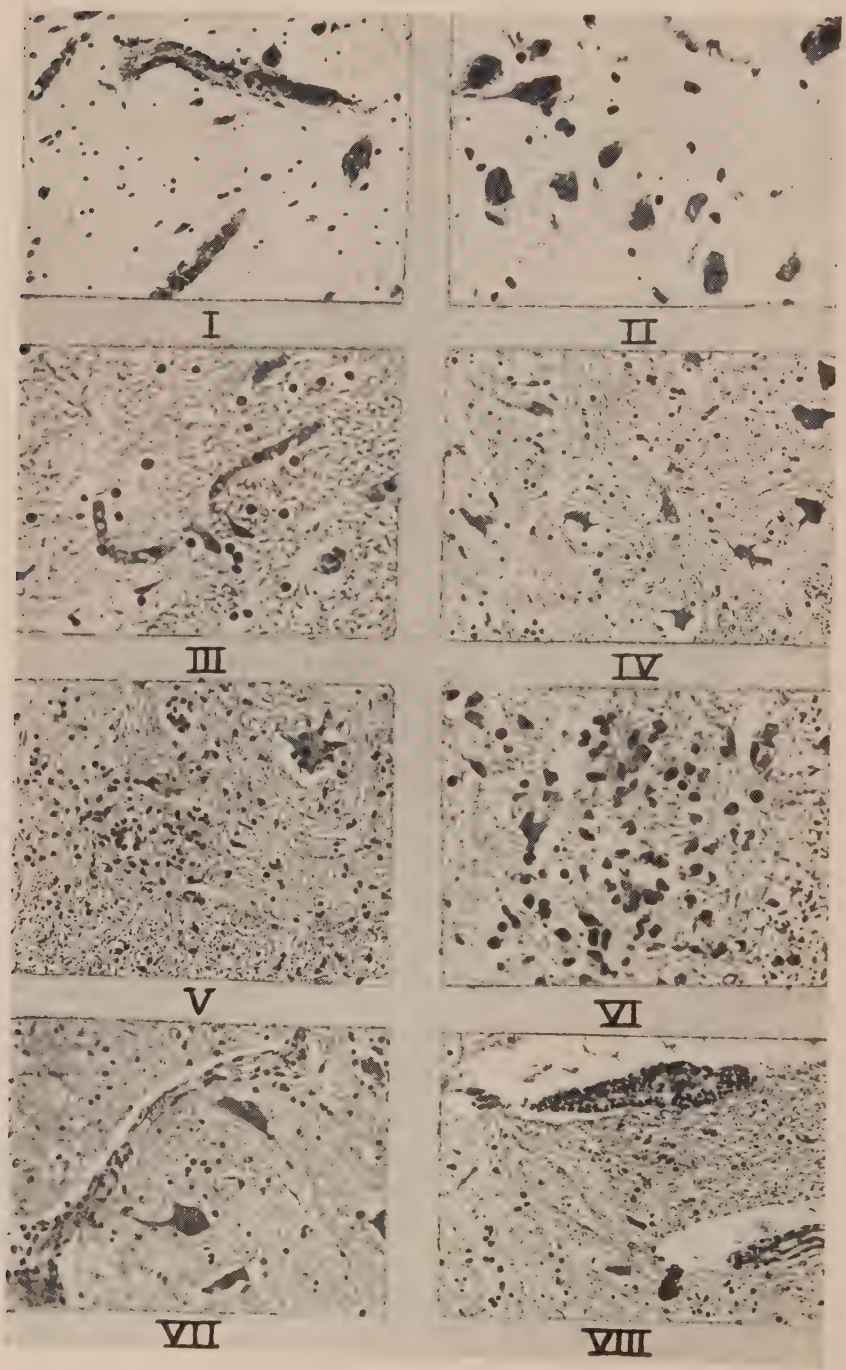
The addition of paratyphoid colon bacillus filtrate (previously termed enteric toxin) to poliomyelitis virus accelerated the production of the disease.[1] What would happen if monkeys *(Macacus rhesus)* were injected with this filtrate as well as with a vaccine made from the organisms that produced it? Adult monkeys have shown some agglutinins for the typho-paratypho-colon group, therefore, young monkeys were chosen for this experiment, since their blood serums contained little or no agglutinins for this group of organisms.[2]

Paratyphoid and colon bacilli were grown in 0.5% glucose broth and after 10 days the media were centrifuged, the organisms combined, autoclaved, and were called the vaccine; the supernatant fluid was passed through a mandler N and W filter and called the P.C.B. filtrate (enteric toxin). Massive doses of the filtrate and vaccine were injected subcutaneously into 3 of 6 baby monkeys within from 2 to 3 months after they had been weaned. The animals, weighing from 890 to 1104 gm., were obtained in September of 1933 and were injected as follows: 10-6-33, 0.25 cc. vaccine; 10-10-33, 0.5 cc. vaccine; 10-17-33, 1.0 cc. vaccine; 10-20-33, 1.0 cc. vaccine; 10-24-33, 1.5 cc. vaccine; 10-30-33, 1.75 cc. vaccine; 11-3-33, 2.0 cc. vaccine; 11-11-33, 2.0 cc. vaccine; 11-20-33, 2.0 cc. filtrate; 11-28-33, 2.0 cc. filtrate; 12-6-33, 3.0 cc. filtrate; 12-13-33, 2 cc. vaccine;

* Expenses defrayed in part by a grant from the Marion R. Spellman Fund, the Cleveland Foundation.

1 Toomey, John A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1015.

2 Toomey, John A., *J. Inf. Dis.*, 1934, **54**, 74.


I
II
III
IV
V
VI
VII
VIII

12-20-33, 3 cc. filtrate; 12-29-33, 2 cc. vaccine; 1-8-34, 2 cc. filtrate; 1-15-34, 2 cc. vaccine; 1-22-34, 3 cc. filtrate; 2-13-34, 2 cc. vaccine. Only a short rest of 2 weeks was allowed before the experiment was started.

Set I. Monkey A, injected as noted above, weighing 1360 gm., and monkey B, control, weighing 1177 gm., were each injected subserosally at multiple points with 25 cc. of a 1% suspension of poliomyelitis virus. Monkey A became very sick immediately after the operation, was slightly better the next day, but began to have spasms of the left side on the 4th day and died on the 5th day. Monkey B, the control, withstood the operation very well, did not show any symptoms and remained active until the 10th day when it developed some weakness of the right leg which became obviously atrophied by the 20th day.

Set II. Monkey C, injected as noted, weighing 1654 gm., and monkey D, control, weighing 1688 gm., were injected in the same manner as those animals of set I. Monkey C became very sick immediately following the operation, was better on the 3rd day, but had a relapse on the 5th day with tremor and weakness of the flexors of the right foot and left hand. This weakness gradually progressed to quadriplegia and death on the 12th day. Monkey D, control, became very ill on the 3rd day following the injection. There was furring and a tremor on the 4th day and weakness of the quadriceps of the right thigh and left foot on the 5th day. It made a complete recovery, save for some atrophy of the left foot, leg and thigh.

Set III. Monkey E, injected as noted, weighing 1452 gm., and monkey F, control, weighing 1422 gm., were each given 80 cc. of a 1% suspension of poliomyelitis virus into a section of the small intestine that had been clamped off. Monkey E died the day following the injection. Monkey F, control, was lively and active until the 9th day when it developed some weakness of the right foot and leg and left hand. On the 20th day, it had an obvious paresis and atrophy of the muscles of the right leg.

A control animal, weighing 1620 gm., was later injected with the

FIG. I. Monkey A-H and E-133 X. Showing capillary increase.
FIG. II. Monkey A-H and E-266 X. Showing ganglion cell damage.
FIG. III. Monkey E-H and E-133 X. Showing capillary increase.
FIG. IV. Monkey E-H and E-133 X. Showing ganglion cell damage.
FIGS. V and VI. Monkey E-H and E-133 X and 266 X. Showing localized area of inflammation.
FIG. VII. Monkey C-H and E-266 X. Showing dilated vessels and damaged ganglion cells.
FIG. VIII. Monkey C-H and E-133 X. Showing typical perivascular infiltration of round cells.

same dosage of filtrate and vaccine and was sacrificed. Histological sections of the central nervous system showed no positive findings.

All 3 of the animals previously injected with the vaccine and filtrate died. All 3 controls developed localized paresis or paralysis, but none died.

Histopathological examination of the cord of animal A which died on the 5th day after the injection of the virus showed degeneration of the anterior horn cells and only a slight inflammatory reaction. There was definite dilatation of the capillaries, but no distinct cuffing. The cord of animal E which died the day after the operation and injection, had massive degeneration of the anterior horn cells with some autolysis, hardly a ganglion cell in the lumbar area escaping some damage. An occasional polymorphonuclear cell and a few glial cells were also found. There was a dilatation of the capillaries, but no distinct cuffing. The sections made from the cord obtained from animal C which died on the 12th day post-injection, were decidedly typical, since examination showed neuronophagia, tremendous glial reactions, an increase in cuffed capillaries, round cells throughout the field together with an occasional leucocyte, and, in certain sections, practically a total destruction of the anterior horn cells.

The production of massive abscesses after subcutaneous injections of staphylococcus, our laboratory culture No. 35, (1 animal with control), 2 successive subcutaneous injections given 2 days apart of the 5th immunizing dose of scarlet fever streptococcus toxin (Dick) (1 animal with control), the presence of massive pulmonary tuberculosis in 11 of the monkeys used during the past 3 or 4 years, and the injection of vaccinia virus intradermally (1 animal with control) did not accelerate the production of poliomyelitis in these animals when they were later given the disease experimentally.

Injections of massive doses of P.C.B. filtrate and vaccine subcutaneously rendered the monkey less immune so that when poliomyelitis virus was later introduced by way of the gastrointestinal tract, the production of the disease was accelerated.

7892 C

Differential Reactions Between Carotene and Oils Rich in Vitamin A.

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It has recently been demonstrated that carotene* gives characteristic color reactions with trichloracetic acid,[1] with chloral hydrate[1] and with a reagent containing a mixture of sulphuric acid and formaldehyde.[2] Tested on the rat, carotene in a biologic sense behaves in a manner similar to vitamin A. From a chemical standpoint, carotene shows a striking resemblance to vitamin A. One molecule of vitamin A is equivalent to half a molecule of carotene. Carotene and vitamin A both yield a characteristic blue color with antimony trichloride. The blue chloroform reaction mixture obtained with carotene in the presence of antimony trichloride yields, however, an absorption band at 590 mu, while the reaction mixture with vitamin A yields a wider absorption band at 610-630 mu.

Recently Rosenthal and Erdelyi[3] have demonstrated the possibility of distinguishing between carotene and vitamin A by applying the antimony trichloride test in the presence of pyrocatechol. They observed that when oil rich in vitamin A or vitamin A concentrate is dissolved in absolute chloroform and heated with antimony trichloride reagent and 0.5% pyrocatechol in chloroform solution, the blue changes to a purple or violet-red color comparable with the one characteristic of an aqueous solution of potassium permanganate. More recently Rosenthal and Erdelyi[4] have demonstrated that in addition to pyrocatechol, hydroquinone, quaiacol and veratrole may be employed. Andersen and Levine[5] have studied their procedure and report positive tests with antimony trichloride with vitamin A-

* The carotene used was obtained from the S.M.A. Corporation, Cleveland, and consisted of a mixture containing a very large quantity of β-carotene and a small quantity of α-carotene.

1 Levine, V. E., and Bien, G. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 335.

2 Levine, V. E., and Bien, G. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 335.

3 Rosenthal, E., and Erdelyi, *J. Biochem. Z.*, 1933, **267**, 191.

4 Rosenthal, E., and Erdelyi, *J. Biochem. Z.*, 1934, **271**, 414.

5 Andersen, A. C., and Levine, V. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 737.

rich oils in the absence of pyrocatechol or any other phenol. They observed that pyrocatechol is even detrimental, since it inhibits the reaction and diminishes its sensitivity.

Using carotene, and haliver oil and cod liver oil as sources of vitamin A, we have compared the action of formaldehyde-sulphuric acid reagent, the trichloracetic acid reagent and the chloral hydrate reagent. Since the above reagents also react with sterols, we have included in Table I the results obtained with ergosterol and with cholesterol.

TABLE I.

Reagent	Carotene	Haliver Oil	Ergosterol	Cholesterol
Formaldehyde Sulphuric Acid	purple ring	bright red in acid layer, blue to purple in chloroform layer	red layer above, with acid layer below showing green fluorescence	cherry pink above, with green fluorescence in acid layer below
Trichloracetic Acid	blue	brilliant blue, changing to purple on heating	red changing to blue, the blue unaffected by heat	no color, pink on heating
Chloral Hydrate	blue	brilliant blue, changing to purple	red to greenish blue to final blue, the blue unaffected by heat	slight pink changing to red

The trichloracetic acid reagent contains 9 parts by weight of crystallized acid and 1 part of water. Three drops of this reagent were mixed with 1 cc. of a chloroform solution. The chloral hydrate (0.5 gm.) was liquefied on the water bath and 1 to 2 drops of a chloroform solution of carotene or oil added, together with a drop of concentrated hydrochloric acid. The formaldehyde-sulphuric acid was made fresh by the addition of one volume of 37 to 40% pure formaldehyde to 50 volumes of concentrated sulphuric acid. Equal volumes of chloroform solution of carotene or oil were used in order to carry out the test.

It can readily be seen that trichloracetic as well as chloral hydrate serve to differentiate carotene, vitamin A-rich haliver oil, ergosterol and cholesterol. The more or less permanent blue color, characteristic of carotene treated with chloral hydrate or trichloracetic acid reagent is changed to an evanescent blue and a more stable purple when heat is applied to a mixture containing vitamin A.

The characteristic tests are obtained with 0.001 mg. of haliver oil representing 0.05 units of vitamin A. Ergosterol may be differen-

tiated from carotene and vitamin A because of the fact that an initial red appears in the reaction mixture and a final blue. Cholesterol reacts but slowly with trichloracetic acid, forming no color at room temperature, but a slight pink on heating. With chloral hydrate heat is applied in order to liquefy the reagent, and the reaction in the presence of cholesterol leads to the development of a slight pink to red color. The formaldehyde-sulphuric acid reagent differentiates carotene from vitamin A, and carotene and vitamin A from cholesterol and ergosterol, but not ergosterol from cholesterol.

The trichloracetic acid reagent and the chloral hydrate reagent have been tested out with chloroform solutions of cod liver oil, butter, wheat germ oil, olive oil, cotton seed oil, linseed oil and sesame oil. The results are shown in Table II.

TABLE II.

Oil Tested	Reagent		
	Trichloracetic Acid		Chloral
	In the cold	On heating	
Cod liver oil	purple	purple	purple
Butter	light purple	light purple	light purple
Wheat germ oil	light gray	gray with definite purple tinge	brown
Olive oil	no coloration	no coloration	muddy orange tinge
Cottonseed oil	light orange	muddy green	brown
Almond oil	blue on standing	blue changing to pronounced green	light brown
Linseed oil	muddy brown	bluish black to black with slight purplish tinge	brown
Sesame oil	salmon pink	reddish brown	brown

Cod liver oil and butter yield an immediate purple when treated with trichloracetic acid or with chloral hydrate. The reaction is much stronger for cod liver oil than for butter. Wheat germ and linseed oil give evidence of a purple tinge on heating with trichloracetic acid, but no indication of the presence of any vitamin A with the chloral hydrate reagent. Wheat germ is known to contain traces of vitamin A.

Summary. Carotene (provitamin A) can be differentiated from vitamin A-containing oils by means of several chemical reagents. Antimony trichloride, trichloracetic acid and chloral hydrate each yield with carotene and with halibut liver oil a characteristic blue color. The blue color persists when the reaction mixture containing carotene is heated on the water bath. Heat, however, transforms the color of the reaction mixture containing halibut liver oil from blue to purple. With cod liver oil and with butter fat the trichlor-

acetic acid reagent or the chloral hydrate reagent yields without the aid of heat an immediate purple.

A reagent containing sulphuric acid and formaldehyde also serves to differentiate carotene from vitamin A-bearing oils. With carotene a purple zone is formed; with halibut liver oil a bright red is developed in the acid layer and a blue to purple in the chloroform layer.

Trichloracetic acid and chloral hydrate also serve as reagents to distinguish between carotene, vitamin A-rich oils, ergosterol and cholesterol. The formaldehyde-sulphuric acid reagent differentiates from one another carotene, vitamin A and the sterols, cholesterol or ergosterol, but does not distinguish ergosterol from cholesterol.

7893 P

Intestinal Motor Inhibition by Parasympathetic Drugs.

F. D. MC CREA AND DONALD F. MARION. (Introduced by W. A. Perlzweig.)

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It was noted by Bernheim[1] that relaxation usually occurred when pilocarpine was added to a strip of guinea pig intestine contracted by histamine. This was confirmed[2] and several instances were noted in which abrupt relaxation occurred when pilocarpine was added to a strip of guinea pig intestine contracted by physostigmine. We found also that in most instances when the intestine was tonically contracted by pilocarpine, it was relaxed by physostigmine. This result was obtained in 4 of 7 trials using the duodenum and jejunum. The investigation has been extended to other parasympathetic drugs using the same method, *viz.*, suspension of the strip in Ringer's solution.

On adding pilocarpine to the bath containing an intestinal strip contracted by acetyl choline, we obtained relaxation of the duodenum, jejunum, ileum, proximal, mesial, and distal colon. In only 3 of the 66 trials was a motor effect produced by the subsequent addition of the second parasympathetic drug (pilocarpine). This effect is only occasionally reversible, that is, acetyl choline relaxes the intestine contracted by pilocarpine only in a minority of the trials. In each intestinal strip tested acetyl choline caused a con-

[1] Bernheim, Frederick, *J. Pharm. and Exp. Ther.*, 1931, **43**, 509.

[2] Craven, Jean D., and McCrea, F. D., *J. Pharm. and Exp. Ther.*, 1934, **51**, 421.

siderable tonic contraction and the increased tonus was usually maintained.

In the case of the esophagus and cardia, Carlson, Boyd and Pearcy[3] suggest that the inhibition resulting from direct or reflex stimulation of the vagus nerve is due to the existing state of tonus. While this hypothesis would explain some of our results, a great many of them are not consistent with it, and we are forced to conclude that another factor is involved. We are continuing these experiments to obtain further data.

Tables I and II summarize the results of the inhibitory effect of pilocarpine.

TABLE I.

Intestine Contracted by Acetyl choline	No. Trials	Effect of Pilocarpine No. Relaxed	No. Contracted	No. No effect
Duodenum	19	17	1	1
Jejunum	21	16	0	5
Ileum	11	8	1	2
Distal colon	8	6	1	1
Proximal colon	4	3	0	1
Mesial colon	3	3	0	0

TABLE II.

Intestine Contracted by Physostigmine	No. Trials	Effect of Pilocarpine No. Relaxed	No. Contracted	No. No effect
Duodenum	4	4	0	0
Jejunum	3	3	0	0
Ileum	4	3	0	1
Distal colon	3	2	0	1

7894 C

Use of Platform Method of Growth in Demonstrating Pigments of Certain Pathogenic Fungi.*

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The platform method of growth has been described.[1, 2] A piece

[3] Carlson, A. J., Boyd, T. E., and Pearcy, J. F., *Arch. Int. Med.*, 1922, **30**, 407.

* Contribution No. 49 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

[1] Williams, John W., *Science*, 1934, **80**, 2064.

[2] Williams, John W., *Arch. Dermat. and Syph.*, in publication.

of blotting paper is bent so that there is a horizontal portion which will just fit into a test tube with 2 legs to act as supporting members. The liquid medium is added almost to the horizontal portion. The tubes are plugged with cotton and sterilized. The plant is made on the horizontal portion. The 2 legs keep the plant supplied with moisture and nutrient by absorption. Care must be taken that the blotting paper contains no substance inhibitory to the organism.

In these experiments the organisms were allowed to grow 6 weeks. The following tubes were planted: pulpit tubes with 1% peptone, 4% dextrose and 1% asparagin, 4% dextrose; broth tubes with 1% peptone, 4% dextrose and 1% asparagin, 4% dextrose. The pH was adjusted to 5.6. Growth took place at room temperature in diffused light.

The organisms studied were: *Achorion schoenleinii, Epidermophyton cruris, Epidermophyton rubrum, Indiella americana, Microsporon felineum, Monosporon apiospermum, Trichophyton granulosum, Trichophyton gypseum asteroides, Trichophyton gypseum lacticolor, Trichophyton niveum, Trichophyton purpureum, Trichophyton sulfureum.*

Achorion schoenleinii. Asparagin medium. In broth there is a small mycelial, intact growth, continuous with the sunken plant; this growth is white and on its surface is a shredded appearance resembling poor grade cotton; all growth is submerged and there is slight milkiness of the broth. On the platform there is sparse growth.

Peptone medium. On broth there is a wrinkled, pellicle of light tan covered with sparse powder; the pellicle tends to form irregular small cones at the medium centrally; there is a small bit of fuzzy mycelium in the bottom of the tube; the medium is amber colored. On the platform is a low sparse growth, very little extending down the stilts and a brown color where the mycelia stick to the glass of the tube; there is no growth in the broth, but the medium is somewhat lighter than the control.

Epidermophyton cruris. Asparagin medium. On broth is a floater most of which is submerged, white with cottony bits hanging as roots in the broth. On the platform is a low, sparse white mycelial growth extending only a short way down the stilts.

Peptone medium. On broth is a white pellicle from which hangs a loose, slightly layered mycelium becoming looser and less as the sunken plant at the bottom of the tube is approached; there is a suggestion of amber at base and sunken mycelium. On the platform is a low mycelial growth extending down the stilts to the medium; where the growth touches the glass is a light caramel

tint; this tint is imparted slightly to the medium; there are no growths in the liquid. On peptone the medium with growth becomes lighter than the control.

Epidermophyton rubrum. Asparagin medium. There is a loose fuzzy, white mycelial growth (some portions slightly tinted pink) on the platform extending a short way down the stilt but not to the medium; there is a reddish pink color at the base and where the mycelia adhere to the glass; there is no growth in the medium. On broth the growth is loose, mycelial, shows a tint of yellow at the pellicle base, shows slight layering and extends from a sunken plant to a mycelial pellicle on the surface.

Peptone medium. On the platform is a mycelial growth with a port wine base which extends down the stilt, attempts to form a pellicle at the medium where it may show a light brown base; there is variation in the mycelia, some being coarse and as port wine colored spicules; there may be colored frosting on the platform; when the mycelia are coarser it seems the color imparted is more a port wine and when the mycelia are finer and whiter this color shows a tendency to browning; where there is more browning there tends to be more powderiness. On broth the growth is loose, mycelial and somewhat layered, apparently grown up from the sunken plant to form a downy pellicle; there is pigment of a light yellow at the base of the pellicle.

Indiella americana. Asparagin medium. In broth there is a bit of growth of a verrucose-like nature at the bottom of the tube. There is practically no growth on the platform but a few small balls of growth at the bottom of the tube.

Peptone medium. On broth a somewhat dense mycelial growth extends from a sunken plant ⅓ way up the medium; the surface of the growth seems as though powdered with a light colored cinnamon; at its edge are very fine rootlets of mycelia adherent to the glass. On the platform is sparse mycelial growth; this growth stains the paper a light grayish brown.

Microsporon felineum. Asparagin medium. The growth is a floater from which extends a fine feathery mycelial growth into the medium for a short way; the medium is slightly milky. On the platform is a mycelial growth sending a long coned tongue down one stilt into the medium and holding the broth to the base of the plant proximal to one wall of the tube; there is a caramel color at the base of the growth and where it touches the glass. The medium is slightly lighter than the control.

Monosporon apiospermum. Asparagin medium. On broth there

is loose, white mycelial growth from a sunken plant toward the surface; there is a suggested layering but no color; the growth surface is downy. On the platform the growth is sparse, white, fuzzy, mycelial, extending a short way down the stilt but not to the medium; there is no growth in the medium.

Peptone medium. A mycelial growth fills the broth extending from a sunken plant to form a pellicle which shows slight black in areas. On the platform is a somewhat soft compact white mycelial growth extending down stilt to medium where it forms a partial cone into the medium; a light caramel coloration shows especially where the growth approximates the stilt. The medium is slightly lighter color than the control broth.

Trichophyton granulosum. Asparagin medium. Growth is most extensive in broth at a white, loose mycelial pellicle from which scant, filmy, loose mycelium extends in diminishing amounts to a sunken plant. On the platform is a white, mycelial, downy growth extending only a slight way down the stilt.

Peptone medium. In broth there is a fairly thick mycelial pellicle, pink where the mycelia touch the glass, blending into a light brown at the edge of the base and a brownish green at the base centrally; from the base hangs a bit of very loose mycelia having a greenish hue becoming looser and less extensive as it extends to the sunken plant in the bottom of the tube. On the platform there is thick, soft, downy mycelium extending down the stilts to the medium where there is attempted pellicle formation; where the mycelia touch the glass is a deep brown pigment and where growth touches the medium is a light yellowish-brown pigment; the medium is a shade lighter than the control broth.

Trichophyton gypseum asteroides. Asparagin medium. In broth mycelial growth fills the bottom ⅓ extending from the sunken plant like a powder puff; between sunken plant and upper surface of growth is an irregular band of yellowish-green pigment; on surface of plant and in medium above is a slight pink hue. There is sparse growth on the platform.

Peptone medium. In broth there is a partial pellicle attached to glass on whose surface is white, fuzzy mycelium and at whose base and where attached to the glass a deep brown grey pigment; a tuft of mycelial growth extends from the partial pellicle into the broth where it floats like a fringe; in the medium which is deep amber colored are sparse bits of growth. There is slight growth on the platform which shows brownish to red tinted where it touches the glass; the growth does not extend down the stilts and there is none

in the medium which is faintly darker than the control peptone broth.

Trichophyton gypseum lacticolor. Asparagin medium. On broth is a powdery, low mycelial pellicle with a suggested pink tint; from the pellicle extends loose mycelium decreasing in amount to the sunken plant below; a portion of this mycelium has a faint brown tint. There is a soft downy mycelial growth on the platform extending down the stilts almost to the medium.

Peptone medium. On broth is a bowl-shaped pellicle whose upper surface is light tan, powdery with low mycelium and whose under surface is a brown; a loose mycelium extends from the under surface to the sunken plant at the bottom of the tube; the broth is amber color. On the platform is a low mycelial growth extending down the stilts to the medium where a tongue and cone are formed to the medium; on the horizontal portion is a pink powder; the medium is a shade darker than the control.

Trichophyton niveum. Asparagin medium. On broth a loose, fluffy growth extends from almost the surface to a yellow tinted sunken plant below. A white mycelium covers the horizontal portion of the platform and extends a short way down the stilt.

Peptone medium. On broth is a thick white mycelial pellicle; where the mycelia touch the glass is a pink tint; at the base and where the medium touches the plant is a caramel color; the pellicle sends a tongue into the medium; this tongue is wet by the medium on one side and mycelial on the other; a small amount of mycelial growth is attached to the sunken plant below. On the platform is a profuse soft white mycelial growth extending down the stilt to the medium where a cone and attempted pellicle is present. There are a few balls of fluffy mycelial growth in the medium which is a shade lighter than the control; where the mycelia touch the glass above is a brown pigment.

Trichophyton purpureum. Asparagin medium. On broth there is a loose, mycelial growth extending upward from the sunken plant below but not reaching the surface, its upper surface is slightly shaggy. The platform shows sparse growth.

Peptone medium. The growth on broth comes from the sunken plant, is loose, hair-like mycelial and continuous with itself; this growth gradually reaches the surface where it forms a white pellicle with a yellow base. On platform is a white soft mycelial growth extending down the stilts to the medium where it grows across as a pellicle; where the growth touches the glass and at its

base is a port wine color; there is no growth in medium; the medium is a shade darker than the control.

Trichophyton sulfureum. Asparagin medium. On broth there is a small amount of cottony growth sediment. On the platform the growth is sparse.

Peptone medium. On broth is a powdery white pellicle whose base is a light amber and from which hangs a loose friable mycelium extending to the sunken plant below; a light amber tint is present especially to areas of the growth. On the horizontal portion of the platform is a soft, sparse white growth, brown where the mycelium touches the glass; the broth is faintly darker than the control.

This method of growth is important whether or not there is a soluble pigment. If so, the platform can be removed and the pigment recovered. If the pigments are not water soluble the broth will be the color of the control broth which is kept for comparison. The morphology of the growths is mentioned here but is covered elsewhere in detail.[2]

In the instance of non-water soluble pigment the growth can be stripped from the blotting paper and the colored blotting paper used for comparison and testing of the pigment with reference to change in color to reaction, etc. The pinks which indicate an increase in alkalinity, since they brown on adding HCl, are changed in color by autoclaving but left less harmed by 10% formalin.

It will be noted that some broths with growths are lighter than the controls. Without further evidence the significance of this observation is not apparent.

This method of bringing out color is considered valuable from the point of view of contrast, as a method of preserving the color for later comparison, as a method of recovering pigment for testing, as a means of determining relative color production on different media, in determining reaction change in media with growth where the color varies with pH and as a method of organismal differentiation. Another possible application is where a colored ingredient is a part of the culture medium. If this is selectively utilized by the organism one should expect the color intensity of the medium due to this substance to decrease.

7895 C

New Heat-Stable Agglutinogens in the Suipestifer Group.

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Observations on absorption of phage by heat-killed bacilli particularly those on the typhosus-paratyphosus B group and some strains of suipestifer suggested that further investigations along this line might yield information revealing new relationships in this highly complex group of organisms.[1, 2, 3] We here report the existence of differences in the suipestifer group first detected by characteristic phage absorption effects and confirmed by subsequent immunization and experiments on agglutinin absorption.

The observations were made with a phage derived from chicken stool filtrates and propagated with a particular American strain of suipestifer, No. 26* in our collection. This phage lysed to a high titer numerous smooth cultures of the biochemically different types, namely American, Kunzendorf and one out of 4 strains of the Hirschfeld type, the other 3 being comparatively resistant. As already reported,[1, 3] the strain Glässer-voldagsen was completely resistant even to the action of undiluted phage. We have since found one culture, No. 92 (American*) in our collection which was poorly lysed but differed from other suipestifer strains in failing to absorb actively from the suipestifer 26 phage, particularly from some passages. Somewhat similar effects were observed with strains 80 and Glässer-voldagsen.

Curiously enough, the same cultures, 80 and 92 were 2 of several strains, mostly of the Kunzendorf type, which on repeated occasions absorbed small quantities of the phage for paratyphosus B in con-

[1] Levine, Philip, Frisch, A. W., and Cohen, E. V., *J. Immunol.*, 1934, **26**, 321.

[2] Levine, Philip, and Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 339.

[3] Levine, Philip, and Frisch, A. W., *J. Inf. Dis.*, 1935, in press.

* For strain No. 26, we are indebted to Dr. Landsteiner, who received it originally from the British National Type Culture Collection. From the same source we obtained the Hirschfeld cultures. Suipestifer No. 80 is in the American Type Culture Collection and is recorded in their catalogue as No. 800; it was isolated by Shaw[4] from the blood stream of a patient with clinical influenza. In our hands this strain produced H_2S. Dr. Ten Broeck[5] supplied us with culture No. 92 which he isolated from a pig.

[4] Shaw, F. W., *J. Lab. and Clin. Med.*, 1926, **12**, 141.

[5] Ten Broeck, C., written communication.

TABLE I.

Absorbed with heat-killed organism No.	Test-organism—suipestifer 26 Dilutions of Anti-suipestifer 26 phage						Test-organism—paratyphosus B 16 Dilutions of Anti-paratyphosus B 16 phage				
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
26 A	+±	++++	++++	++++	++++	+++±	cl	cl	cl	cl	+
84 A	+±	++++	++++	++++	++++	+++±	cl	cl	cl	cl	+
100 H	++++	++++	+++±	++++	++++	+++±	cl	cl	cl	±	++
63 GV	tr	+±	+++	++++	++++	+++±	cl	cl	cl	±	++++
80 K	cl	++	++++	++++	++++	+++±	cl	+±	+++	++++	+++±
92 A	cl	cl	cl	++	++++	++++	cl	+	+±	+++±	++++
16	cl	cl	cl	++	+++	++++	++++	++++	+++±	++++	+++±
Unabsorbed	cl	cl	cl	tr	+±	++++	cl	cl	cl	cl	tr

Culture No. 16 is paratyphosus B; all other cultures are in the suipestifer group.
A = American; H = Hirschfeld; GV = Glässer-voldagsen; K = Kunzendorf.
The turbidity reading recorded is that made 6 hours following the addition of the test organisms; cl indicates complete clearing; ±, +, +±, ++, etc, indicate increasing degrees of turbidity.

trast to the other suipestifer strains which did not at all absorb. These effects are indicated in Table I.

In view of these results, it seemed desirable to determine whether serological characteristics could be found to correspond more or less with the qualitative differences shown in phage absorption.

Accordingly rabbits were immunized intravenously with increasing quantities of boiled bacillary suspensions of several varieties. Specimens of sera obtained after 3 and 6 injections were tested both by direct titration and by absorption. The absorptions were made with sera diluted 1 :100; the bacilli employed for the absorption were previously heated for one hour at 80°C., while the suspensions used in the tests for the quality of the absorbed sera were previously treated with absolute alcohol to prevent the action of flagellar agglutinins, if present. In Table II is recorded a typical experiment with sera vigorously absorbed with an excess of bacterial bodies.

TABLE II.

Rabbit Serum No.	Absorbed with Strain No.	Tested with alcohol-treated suipestifer cultures, No. 26A	84A	100H	63GV	80K	92A
413 (anti-100)	84	0	0	±	+±	+±	+±
	92	+++	+++	+++	++	0	0
426 (anti-92)	84	0	0	+	++	++	++±
	92	0	0	0	0	0	0

The results indicate that suipestifer Nos. 80 and 92 form one sub-group, whereas the great majority of the American type (represented in Table II by Nos. 26 and 84) and Kunzendorf strains (not recorded in the table) form a second sub-group. Apparently Gläsֵser-voldagsen and perhaps also suipestifer 100 (Hirschfeld) have still other properties. It is interesting to note that sera for strain 100 contain several types of qualitatively different antibodies, whereas most other cultures of the American or Kunzendorf types fail to produce an antibody specific for 80 and 92. Such an antibody was found to be present in each of 3 rabbits injected with suipestifer No. 92 and also 100. Direct titration of these sera did not readily reveal the relationships described in the agglutinin absorption tests.

Experiments are under way to determine whether or not the strains 80 and 92, in contrast to others are capable of specifically absorbing small quantities of the paratyphosus B antibody.

In view of the relationship of newport to the suipestifer group, it became necessary to re-examine this question. Our preliminary ex-

periments showed that all actively agglutinating sera except those produced by strain 92, agglutinated newport also, and, in harmony with this result, all suipestifer cultures except 80 and 92 removed this agglutinin.

7896 P

Polyvalency Demonstrated by Antiphages.

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A general procedure was described for the study of the specificity of absorption of bacteriophage by heat-killed bacilli.[1, 2, 3] Polyvalent phages derived from chicken stool filtrates and propagated either against a strain of paratyphosus A or enteritidis were shown to consist of at least 2 prominent qualitatively different fractions, the one selective for suipestifer strains and another for the typhosus-paratyphosus B group. This effect was readily demonstrated by testing the quality of the residual phage with several serologically different sensitive organisms. The question naturally presented itself as to whether or not antiphages may be employed to indicate the presence in a phage of the several fractions.

Accordingly 4 series of rabbits were injected intravenously with the 2 polyvalent phages for paratyphosus A and for enteritidis and also with the presumably monovalent paratyphosus B and suipestifer phages. After 4 injections of 2 cc. each, potent antiphages, practically lacking in agglutinins were readily obtained in all instances except in the case of the suipestifer phage.

Experiments demonstrating the production of 2 antibodies corresponding to the fractions in the polyvalent phages are given in Table I. The results show that the antisera for the paratyphosus A and enteritidis phages contain potent antibodies which neutralize the action of the typhosus-paratyphosus B fraction of the polyvalent enteritidis phage (Table I A, aertrycke of the paratyphosus B group, as test organism); both antisera also possess antibodies for the suipestifer fraction but to varying degrees of activity. Although

1 Levine, Ph., Frisch, A. W., and Cohen, E. V., *J. Immunol.*, 1934, **26**, 321.
2 Levine, Ph., Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 339.
3 Levine, Ph., Frisch, A. W., *J. Inf. Dis.*, 1935. In press.

TABLE I.
Dilutions of Enteritidis Phage.

Phage antisera diluted 1:50	A. Test organism—aertrycke 10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	B. Test organism—suipestifer 10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Paratyphosus B	+±	+++	++++	++++	++++	cl	cl	cl	cl	++++
Paratyphosus A	+	+++±	++++	++++	++++	++++	++++	++++	++++	++++
Enteritidis	+	+++	++++	++++	++++	cl	cl	±	+++	++++
Dysenteriæ Shiga (bacillary)	cl	cl	tr	+±	++++	cl	cl	cl	cl	+++

0.5 cc. of given dilutions of phage were mixed with 0.5 cc. of the various sera diluted 1:50; incubation overnight at 37° C.; the following day 0.5 cc. was removed from each of the tubes of the entire set into another series of tubes (B). To series A and B was added 4.5 cc. broth and the test organisms indicated in the table. The turbidity reading recorded was that made 6 hours after the addition of the test organism; cl indicates complete clearing; tr, trace; ±, +±, ++, etc., indicate increasing degrees of turbidity.

the antiphage for paratyphosus B contains just as potent neutralizing antibodies as the other 2 antiphages, the former has no inhibiting action whatever on the same phage when suipestifer is used as the test organism. (Table I B.)

A result practically identical with that shown in Table I A, was obtained when these sera were tested in the same manner with a phage for paratyphosus B even when a strain of typhosus was used as test organism.

The experiments are of general interest from 2 points of view. First, they suggest, as is to be expected, that similar specificities are exhibited by the reaction of phage with the heat-killed bacilli on the one hand and with antiphage on the other.[4, 5] Second, the use of polyvalent phages with qualitatively specific fractions makes it feasible to study antiphage reactions in such heterologous combinations of reagents as will exclude or minimize interfering agglutinin reactions which may, at times, result from injection of phage containing filtrates. Thus, in our experiments the paratyphosus A antiphage inhibits the action of the enteritidis phage when suipestifer is employed as a test organism. (Table I B.)

In our opinion, any attempts to classify phages on the basis of specific neutralization must take into account the fact that phages may radically differ in their composition and yet share a particular fraction in common.

7897 C

Colony Differences in Survival of Adrenalectomized Rats.

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(Introduced by W. W. Swingle.)

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There has been much disagreement among investigators concerning the life-span of the rat after adrenalectomy. While the majority of workers prior to 1930 found that 50% or more of their rats survived adrenalectomy, due to the presence of accessory cor-

[4] Burnet, F. M., *Brit. J. Exp. Path.*, 1933, **14**, 93; *J. Path. and Bact.*, 1933, **36**, 307.

[5] Andrewes, C. H., and Elford, W. J., *Brit. J. Exp. Path.*, 1933, **14**, 367.

tical adrenal bodies, several more recent workers[1-4] have reported that all or nearly all of their animals died after adrenal removal. Gaunt[5] offered evidence indicating that these inconsistent results may have been in part due to inherent colony differences in the susceptibility to adrenal removal. This report offers further evidence for such colony differences.

Dr. J. T. Lewis of the Instituto de Fisiologia, Buenos Aires, one of the investigators to find high percentages of survival after adrenalectomy, sent a group of his rats to Dr. W. W. Swingle of Princeton University in the care of Dr. De Meio. He requested that they be adrenalectomized and their survival compared to colonies we had previously studied. Professor Swingle sent these animals to us to conduct this study.

To eliminate variable factors due to care, diet, climate, etc., none of the original animals obtained from Dr. Lewis' colony were used in these experiments, but only the offspring, born and raised in our laboratory, in the same room with our animals.

Operative technique and care were as previously described.[5] Considerable of the adrenal pedicle and adjacent fat were removed, although this precaution does not apparently influence our survival results.

Survival Results—Mature Animals. Thirty-seven animals of the Lewis strain, varying in weight from 148 to 236 gm., averaging 192 gm., were operated. Of the 37, 2 deaths occurred on the 14th day, 2 on the 18th day, and one death on each of the following days: 21st, 24th, 28th, 32nd, 42nd, 44th, 54th, 74th, and 87th. The total deaths were 13 in number, or 35.1% of the total. The other 24 cases (64.9%) lived in good condition until killed for autopsy from 3 to 4 months after adrenalectomy. (Table I. Lewis Colony—Adults.) These results closely parallel those obtained by Dr. Lewis, himself, who found that from 60 to 80% of his colony survives adrenalectomy.[6] Lascano-Gonzalez[7] reported a 55% survival in this same colony despite the occurrence of an epidemic of bronchial pneumonia.

The highest survival we have ever obtained in an adult control

1 Pencharz, R. I., Olmsted, J. M. D., and Giragossintz, G., *Phys. Zool.*, 1931, **4**, 501.

2 Martin, S. J., *Am. J. Phys.*, 1932, **100**, 180.

3 Firor, W. M., and Grollman, A., *Am. J. Phys.*, 1933, **103**, 686.

4 Evans, H. M., *Memoirs of U. of Calif.*, 1934, **10**, U. of Calif. Press, Berkeley.

5 Gaunt, R., *Am. J. Phys.*, 1933, **103**, 494.

6 Lewis, J. T., *Am. J. Phys.*, 1923, **64**, 503.

7 Lascano-Gonzalez, J. M., *Rev. Soc. Argent. Biol.*, 1933, **10**, 28.

TABLE I.
Survival of Rats from Different Colonies and of Different Ages after Adrenalectomy.*

	Total Mortalities	No. Surviving Indefinitely	Av. Survival in Days, with Standard Errors	Deaths during first 15 Days	Deaths during 15th to 30th Day	Deaths after 30 Days	Day of first death after Operation	Total Cases
Lewis Colony—Adults	13 (35.1%)	24 (64.9%)	36.2 ±6.2	2 (5.4%)	5 (13.5%)	6 (16.2%)	14th	37
Controls—Adults	21 (87.5%)	3 (12.5%)	17.8 ±2.5	9 (37.5%)	9 (37.5%)	3 (12.5%)	2nd	24
Lewis Colony—30-day-old	25 (96.2%)	1 (3.8%)	8.3 ±0.83	23 (88.5%)	2 (7.7%)	0.0	3rd	26
Controls—30-day-old	44 (95.6%)	2 (4.4%)	8.0 ±0.77	42 (91.2%)	1 (2.2%)	1 (2.2%)	3rd	46

*"Lewis Colony" refers to those animals sent by Dr. J. T. Lewis from Argentina.
"Controls" refers to those animals of our colony operated at the same time as those of the Lewis Colony.

series of our own animals, generally showing from 90 to 95% mortality, was in a group of 24 operated at the same time and under conditions identical to those of the Lewis strain. Wide differences were, however, noted between the survival of these 2 colonies as seen in Table I (Controls—Adults).

Survival Results—30-day-old Animals. The frequent use of 30-day-old rats for adrenal experiments makes their survival of interest. Survival differences noticed between the adults of these 2 colonies were not apparent in the young animals, where both groups succumbed rapidly. The young rats were weaned at 28 days of age and adrenalectomized at 30 days.

Twenty-six of the young Lewis colony animals were operated. One survived indefinitely. The other 25 (approximately 95%) died between 3 and 18 days with an average life-span of 8.28 days (Table I).

Forty-six young animals of our colony were operated. Two survived indefinitely. The other 44 (95.5%) died between 3 and 34 days, with an average survival of 8 days (Table I).

Cortical Accessories. In 16 of the 24 adult animals of the Lewis strain which survived, one or 2 accessory cortical bodies in each rat were identified by histological examination. In 4 cases these accessories were distinctly macroscopic in size. In 12 cases the accessory was on the left side, either near the proximal part of the adrenal vein, or on the renal vein, or on a side branch of the adrenal vein. In 4 cases the accessory was found on the right side, near the site of the excised right adrenal, and always very near the posterior vena cava. As mentioned above, enough of the pedicle and tissues surrounding the adrenal were always removed to assure that these accessories were not fragments of the main gland left behind at operation. In only 4 of the 8 cases in which no accessories were found was there a fairly complete search made; because in the others, small macroscopic bodies were mistakenly thought to be adrenals, and no other tissues removed for microscopic study.

Conclusions. These results further indicate that different colonies of rats may differ markedly in their sensitivity to adrenal removal—a fact explaining, in part, the widely different reports concerning the survival of adrenalectomized rats. The logical assumption, we now are testing histologically, is that these colonies differ in the amount of accessory cortical tissue which they possess. However, rats having accessories may succumb to adrenalectomy unless some treatment is given to delay death long enough for hypertrophy of the accessories to occur. This is indicated by the fact that approx-

imately 50% of the adults of our colony survive when treated for a time after adrenalectomy either with cortical extract[8] or with salt,[9] whereas much smaller percentages survive when untreated. The work of Lascano-Gonzalez indicates that in the Lewis colony all of the animals have cortical accessories.

7898 C

Proteolytic Enzyme Content of Latex from the Fig Tree (Ficus Carica L'). Seasonal Variation.*

BENJAMIN H. ROBBINS. (Introduced by P. D. Lamson.)

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tenn.

We have reported[1, 2] the isolation and proteolytic activity of the anthelmintic agent in the sap obtained from numerous species of the fig tree (genus Ficus), from South America, Cuba, and Alabama. The sap from *F. laurifolia* and *F. glabrata,* has been widely used as a general anthelmintic by the natives of South America and Panama and its anthelmintic action confirmed by several medical investigators who have noticed that it was particularly effective against *Trichuris trichiura.*[3-7]

In the present study we have followed the variation in the enzyme content of sap taken from the domestic fig, *F. carica L',* throughout the year. Mrs. E. L. Caldwell, at the International Health Division Laboratory in Andalusia, Alabama, has collected and sent us 8 samples at regular intervals over a 12 month period. The samples were analyzed within 4-5 days after their collection.

One cubic centimeter of the sap is added to a 2% solution of gelatin and a formol titration made immediately on an aliquot portion

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8 Gaunt, R., and Gaunt, J. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 490.

9 Gaunt, R., Tobin, C. E., and Gaunt, J. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 134.

* The funds for carrying out this work were given by the International Health Division of the Rockefeller Foundation.

1 Robbins, B. H., *J. Biol. Chem.*, 1930, **87**, 251.

2 Robbins, B. H., and Lamson, P. D., *J. Biol. Chem.*, 1934, **106**, 725.

3 Caldwell, F. C., and Caldwell, E. L., *Am. J. Trop. Med.*, 1929, **9**, 471.

4 Hall, M. C., and Augustine, D. L., *Am. J. Hyg.*, 1929, **9**, 602.

5 Mouat-Briggs, C. E. F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1914, **8**, 216.

6 Paez, F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1914, **8**, 217.

7 Montoya, T. W., *Trop. Dis. Bull.*, 1922, **19**, 240.

to determine the amount of amino nitrogen present at the beginning of the experiment. Then the mixtures are incubated at 35° for 24 hours and a second titration made. The difference between the 2 determinations gives the increase of amino nitrogen due to the hydrolytic action of the enzyme added, which is indicative of the concentration of the enzyme. Controls, using sap from *F. glabrata,* which had been in the laboratory ice box for 12 months, were run with each test. Table I shows the fluctuation in enzymatic activity during the year as estimated by the liberation of amino nitrogen.

TABLE I.

Mgm. N_2 Liberated by Action of 1 cc. Sap on 100 cc. of 2% Gelatin at 35° for 24 Hours.

Date	Control *F.glabrata*	*F. carica L'.*	Remarks
Feb. 10	55	67.	Sap thick and creamy
April 1	53	71.	
May 1	56	24.	
June 1	53	4.2	Sap thin and watery
July 10		10.	
Aug. 13	54	43.	Sap thick and creamy
Oct. 10†	64	62.	" " " "
Dec. 5	50	66.	

†The temperature of the incubator reached 45° over the night and this accounts for the relatively high rate of hydrolysis.

The amount of each sample of sap was not sufficient for studies on the concentration of solid material present, but it was obvious from visual examination that the samples collected in May, June and July contained very little solids in comparison with the winter samples.

Summary. There is a marked seasonal variation in the amount of enzyme present per unit volume of sap and the concentration is lowest in early summer.

7899 C

Proteolytic Enzyme in the Latex from the Fig Tree (Ficus Glabrata). The pH of Optimal Activity.*

BENJAMIN H. ROBBINS. (Introduced by P. D. Lamson.)

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For many years the sap from various species of the fig tree (genus Ficus) has been used in Central and South America as a vermifuge.[1, 2] It has been shown to have a fair action against Ascaris and *Trichuris trichiura.*[3] We[4] found that the active anthelmintic principle was a proteolytic enzyme that was active over a pH range of 4 to 9 and that it would hydrolyze coagulated egg albumen, gelatin and casein. From the hydrolysis of casein, crystals of tyrosine and 'leucine balls' separate out. Dilute solutions of the sap (0.5 to 1%) will digest the body walls and organs of live Ascaris.

The present paper gives the results of studies on the influence of different hydrogen-ion concentrations upon the proteolytic activity of the enzyme as determined by its action on gelatin.

Preparation of the dry enzyme mixture. The crude sap, a thick creamy mixture, contains about 25% solids, a part of which can be separated by centrifuging (this part is inactive); the remaining or active material is precipitated by 3 parts of acetone and is separated by decanting and centrifuging. The active part is redissolved in water and again precipitated by acetone and dried over $CaCl_2$ in a vacuum desiccator. One hundred cc. of the sap yields about 10-12 gm. of the dried powder to which we have given the name Ficin, from the generic name of the trees from which the active sap is obtained.

For determining quantitatively the enzymatic action on gelatin the formol titration was used. To 100 cc. of a 2% gelatin solution is added the sample of enzyme under study. A formol titration is carried out immediately on a 20 cc. aliquot. The mixture is then incubated at 35° for 24 hours and a second titration made. The difference between the 2 determinations is due to the hydrolysis and is proportional to the activity of the enzyme. Toluene was used as a

* The funds for carrying out this work were given by the International Health Division of the Rockefeller Foundation.

1 Paez, F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1914, **8**, 217.

2 Mouat-Briggs, C. E. F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1914, **8**, 216.

3 Caldwell, F. C., and Caldwell, E. L., *Am. J. Trop. Med.*, 1929, **9**, 471.

4 Robbins, B. H., *J. Biol. Chem.*, 1930, **87**, 251.

preservative. The results are reported in milligrams of amino nitrogen set free by the action of the enzyme upon 100 cc. of 2% gelatin in 24 hours at 35°C.

Seven samples of a solution containing 40 mg. each of ficin were adjusted to pH values of 3 to 9 by the addition of hydrochloric acid or sodium hydroxide. These mixtures were then placed in an incubator at 35° for 3 hours. At the end of this time they were neutralized and added to flasks containing gelatin and diluted to 100 cc. (final gelatin concentration 2%). Formol titration was immediately carried out and again after 24 hours incubation at 35°C. The results of 2 such series are given in Fig. 1. The mixtures in series 2 were incubated 24 hours longer and again titrated and the data shown in curve 3 of Fig. 1.

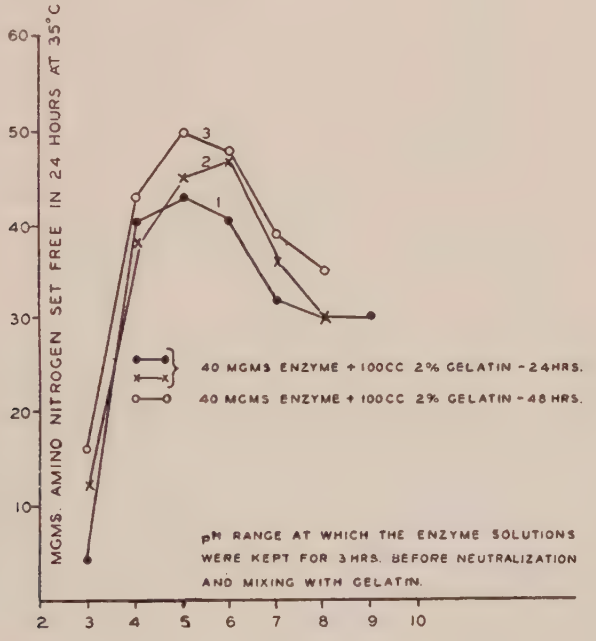

FIG. 1.

Hydrolysis of gelatin by samples of enzyme, obtained from the sap from the fig tree *F. glabrata*, which had been kept at varying pH values from 3 to 9 for 3 hours before neutralization and mixing with the gelatin for digestion.

Seven samples of gelatin (80 cc. of a 2.5% solution) and 7 samples of enzyme (20 cc. each containing 40 mg. of ficin) were adjusted to pHs of 3 to 9 and then mixed. Formol titrations were made immediately upon 20 cc. aliquots and again at the end of 24 hours incubation at 35°. The results of this series and one using 80 mg. of ficin are given in Fig. 2.

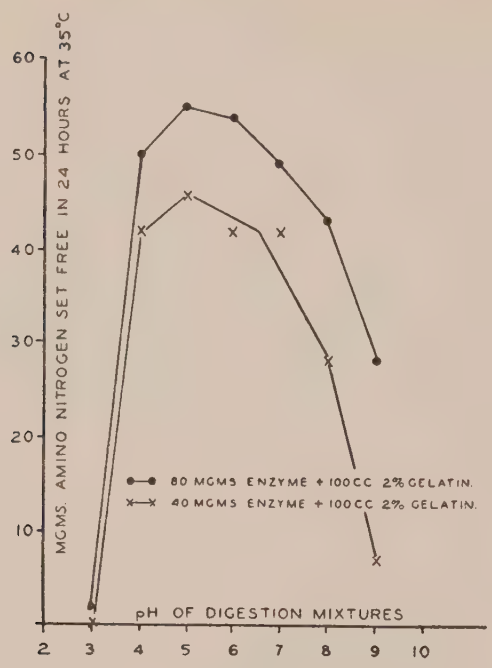

FIG. 2.

Hydrolysis of gelatin mixtures, of pH values from 3 to 9, by the proteolytic enzyme in the sap from the fig tree *F. glabrata.*

It can be seen from the Figs. 1 and 2 that the pH of optimal activity for ficin is 5. That a pH of 3 destroys the enzyme action whereas a pH of 8 is inhibitory, is shown by comparing the curves of Figs. 1 and 2 and also by data obtained when the digestion mixtures used in curve 1 in Fig. 2 were neutralized and incubated for 24 hours longer. There was no increase in the free amino nitrogen in the mixture originally incubated at a pH of 3 whereas in the mixture originally at pH of 8 showed an increase of 13 mg.

Summary. The optimum hydrogen-ion concentration for the ficin-gelatin proteolysis is pH 5.

7900 P

Effect of Diet on Ketonuria in the Rat.

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From the Department of Biochemistry, University of Southern California School of Medicine, Los Angeles.

It was noted[1] that the fasting ketonuria in the rat rarely exceedel 2 mg. per day over periods of several days. More recently, instances of higher values in the excretion of acetone bodies in fasting rats fed sodium chloride solution have become increasingly frequent, so that at least 80% give higher results.

Thus, the average values of acetone bodies for fasting male rats were 7.7 mg. (0.0-45.3) for 34 rats on the first fast day and 12.3 mg. (0.6-27.2) for 11 animals on the second one. The corresponding averages on 19 female animals fasted for 3 consecutive days were respectively 2.8 mg. (0.0-18.8), 10.4 mg. (0.0-33.0), and 9.6 mg. (0.2-35.9). Moreover, it was found that although the same sex difference in the excretion of ketone bodies followed the administration of diacetic acid as was previously observed, the levels in our recent experiments during 5 days of fasting were consistently higher than those previously obtained. The only variation in experimental regime from that earlier employed to which these alterations could be ascribed, was a change in the stock diet. Because of the outbreak of pellagra in our colony, our previous high carbohydrate diet (II) had been fortified in Vitamin G several months earlier by the inclusion of 5% of desiccated liver therein (Diet III). It was found that the fasting ketonuria observed over periods of several days in rats previously on Diet III was gradually reduced to a low level when the animals were returned to Diet II (liver-free) for 60 days. The original level of fasting ketonuria of 4.9 (0.0-17.7) for 9 rats on the first fast day, 17.6 mg. (2.7-29.2) for 8 animals on the second day, and 15.1 mg. (3.5-30.3) for 5 rats on the third one was reduced after 60 days on Diet II to a mean of 0.9 mg. (0.6-1.2) for 9 rats on the first and 1.7 mg. (0.5-3.1) for the same animals on the second fast day.

On the other hand, when rats previously on Diet III were fed on a diet containing 45% of desiccated liver, the level of fasting ketonuria was slightly raised after 31 days. After 50 days the ketonuria reached abnormally high values in 7 of 8 animals used. The average

[1] Butts, J. S., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1933, **100**, 415.

results for 4 males were 26.3 mg. (4.3-50.2) for the first fast day and 60.7 mg. (16.5-89.5) for the second one. The similar high levels which obtained in the 4 females were 10.5 mg. (0.6-22.4) and 46.1 mg. (3.8-113.4) for the 2 fasting days respectively. On one case an excretion of 113 mg. was found, which is the highest level of fasting ketonuria we have ever observed in the rat.

So far we have been unable to prevent the onset of the ketonuria by the administration of choline hydrochloride in doses of 60 mg. per day. The effect of the liver diet is probably to be ascribed to the mobilization of the fat in the liver as indicated by the experiments of Blatherwick *et al.*[2] Comparative studies on the relationship between the level of liver fat and the extent of fasting ketosis after employing diets high in various fats is in progress.

7901 P

Sexual Variation in Carbohydrate Metabolism. VII. Effect of Alkalosis on Fasting Ketonuria in the Rat.

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Booher and Killian[1] first reported that abnormally large amounts of acetone bodies were associated in human subjects with conditions of uncompensated alkalosis which arose either from an excessive alkali administration or from the loss of HCl by excessive vomiting. Butts and Deuel[2] previously showed that no sex differences occurred in the slight ketonuria which occurs in fasting rats previously fed on a high carbohydrate liver-free diet although a definite sex variability was demonstrated in rats to which diacetic acid was administered. In the present tests sodium bicarbonate was fed to fasting rats in a dose of 2.17 mg. per sq. cm. of body surface by stomach tube in 3 divided doses daily. This corresponds with the alkali intake in tests in which sodium acetoacetate was administered in an amount of 1.5 mg. per sq. cm. per day (calculated as acetone). The acetone body excretion with the male rats over a 4-day experimental period

2 Blatherwick, N. R., Medlar, E. M., Bradshaw, P. J., Post, A. L., and Sawyer, S. D., *J. Biol. Chem.*, 1933, **103**, 93.

1 Booher, L. E., and Killian, J. A., PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 528.

2 Butts, J. S., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1933, **100**, 415.

averaged 5.3, 8.6, 5.8, and 6.1 mg. per day for 14 rats on the first day and 11 animals on the other days which corresponds with an excretion of 0.14, 0.24, 0.16, and 0.17 gm. per sq. meter of body surface respectively. The females developed an appreciable ketonuria in distinction to the almost blank values on the males. Thus, the acetone body excretion in the urine gave a mean of 21.3, 39.6, 39.2, and 37.4 mg. of acetone of 14 rats on the first day and 11 on each of 3 following days. The values calculated on the basis of grams per square meter of body surface per day were 0.77, 1.39, 1.39, and 1.32 gm. respectively. A normal response to diacetic acid administration was demonstrated on a control day. The experiments show the greater susceptibility of the female rat to alkalosis than the male.

7902 C

Effects of Short Wave Electric Fields on Cataphoretic Velocities of Streptococci.

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A type of radiotherm developed in the Research Laboratories of the General Electric Company was used for the production of the high frequency fields. The oscillator delivered approximately 0.1 ampere per square inch of plate surface at a frequency of 27.3 million cycles (wavelength 11 meters). Cataphoretic measurements were made at level 0.211 D, where D is the total depth of the flat cataphoretic cell in the Mudd assembly of the Northrup-Kunitz apparatus. Measurements during a long series of experiments indicated a mean potential gradient (v/l) of 7.0 ± 0.4 volts per cm. for the field strength, or driving force, in the center of the cataphoretic cell. The mobility, *u*, is calculated from the relationship:

$$u = \frac{d}{t_c \times (v/l)} = \frac{48.1}{t_c \times 7.0} = \frac{6.87}{t_c}$$

in which t_c is the "cataphoretic time," that is, the time required by the streptococcus to move over a distance "d" under the influence of the electric field of the cell. Throughout these experiments the room temperature was 25°C., with a variation of ±2°C.

The results of measurements of cataphoretic velocity are presented as distribution curves of cataphoretic time. As the cataphoretic time increases there is, therefore, a reduction in cataphoretic mobility and, presumably, in the net charge on the surface of the streptococcus. The distribution curves are based on the measurements of the velocities of 20 organisms of each suspension. In the distribution curves which are given in this paper, the total amount of energy of the high frequency field to which each of the cultures was exposed is proportional to the time stated in minutes above each distribution curve. The number of each suspension, indicating the order in which cataphoretic measurements were made on a series of tubes, is given in a circle to the left of each distribution curve. Subcultures are represented as tenths of the number; that is, 1.1 is the first subculture of streptococci in tube number 1, and so forth.

The details of the methods used in the isolation and preparation of cultures have been presented elsewhere.[1, 2]

When a tube containing 2 cc. of "dextrose-brain broth" was exposed to the high frequency field for a period of 5 minutes, the temperature of the contents of the tube rose 20°C. (from 25°C. to 45°C.). By intermittent application of the high frequency field it was possible to avoid lethal action and to bring the contents of all tubes in a given series to the same maximal temperature, which was approximate to, or slightly less than, the usual body temperature.

In Fig. 1 are shown the results of an experiment with samples of culture number 5829 (streptococcal strain isolated from the nasopharynx of a person in the early stages of acute epidemic poliomyelitis). The 12 sample tubes of this culture were exposed to the field for the respective total doses of 0 (2 controls), ½, 1, 1½, 2, 2½, 3, 3½, 4, 4½ and 5 minutes in cumulative doses of ½ minute each. A complete cycle of changes in cataphoretic distribution occurred during exposure of samples of the culture to the high frequency electric field for a period of 5 minutes. The 2 control cultures exhibited very similar types of distribution, consisting chiefly of streptococci at the time of 4 seconds. The sample of culture which had received 2½ minutes of treatment showed a marked distribution maximum at a cataphoretic time of 3 seconds, indicating a 30% increase in velocity as compared with the control cultures. The tubes at the 3 succeeding dosages showed progressive decre-

[1] Rosenow, E. C., and Jensen, L. B., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 442.

[2] Rosenow, E. C., and Jensen, L. B., *J. Infect. Dis.*, 1933, **52**, 167.

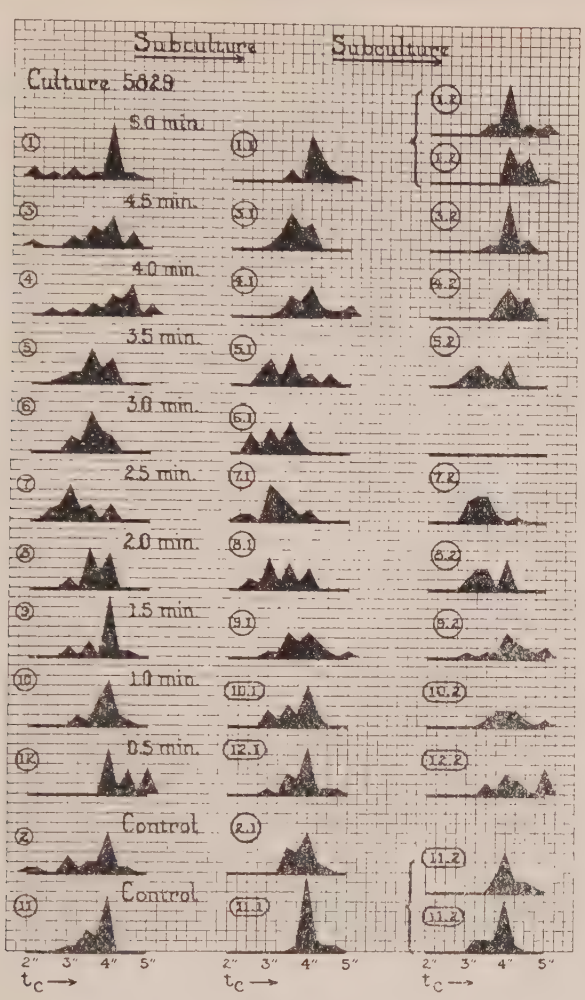

FIG. 1.

Distribution curves of cataphoretic time showing the cyclic shift in cataphoretic distribution produced by the application of a short electric wave field to samples of culture number 5829. The cycle of changes in distribution is maintained in two successive subcultures of each of the samples.

ments in cataphoretic velocity distribution. The tubes which had received exposures of 4½ and 5 minutes, respectively, possessed distribution curves nearly identical with those of the control tubes.

Each of the samples in this series underwent 2 successive subcultures. Although the distribution of each group of organisms in the subcultures is not always identical with that of the parent culture, nevertheless the cycle of changes produced by high frequency

energy in the original series of samples is again evident in the series of subcultures.

In another series of experiments, 5 sample tubes of culture No. 5741 (strain isolated from the nasopharynx of a patient with severe ophthalmic herpes zoster) were exposed to the short electric wave field in cumulative intervals of one minute each for a total of 4 minutes. The control sample of culture showed a distribution with a marked maximum at a cataphoretic time of 4 seconds (1.7 $\frac{\mu/\text{sec.}}{\text{v/cm.}}$); and the tube treated for 2 minutes had a distribution in which the major portion of the organisms were found at a time of 3 seconds ($2.25\frac{\mu/\text{sec.}}{\text{v/cm.}}$); on the other hand the tube exposed for 4 minutes showed a distribution practically identical with that of the control sample of the series.

The changes in cataphoretic distribution following exposure of a sample of culture to the short wave electric field do not appear to be explicable on any basis other than that of a direct effect of high frequency energy either on the streptococcus as a unit or on the surface regions of the organism. However, no information regarding the mechanism underlying the changes produced by high frequency fields is available. The voltage difference between the plates of the condenser, in the region in which the organisms were treated, amounts to about 2500 volts. This would give an electric field of approximately 0.05 volt per micron, alternating in direction about 30,000,000 times a second. The diameter of the streptococcus is approximately 1 micron. We do not know whether there are electric stresses set up in the orientated molecules of the surface of a particle which are sufficient to change the pattern of orientation. The quantum energy of this type of radiation would not appear to be of sufficient magnitude to produce effects such as those we have observed. *Conclusions.* 1. It is possible to produce changes in the cataphoretic distribution of streptococci by means of a high frequency electric field. 2. Successive increments of exposure to short wave electric energy produce cyclic or periodic changes in the cataphoretic distributions of the treated suspensions of streptococci. 3. The changes produced by high frequency fields in the cataphoretic distribution of streptococci are maintained in the subcultures of these organisms.

7903 C

Electrophoretic Characteristics of Streptococci Exposed to High Frequency Fields, and Subsequently Injected into and Recovered from Rabbits.

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Evidence has been presented[1] that the cataphoretic mobility of a culture of streptococci could be changed by application of high frequency energy. These changes were transmitted to the subcultures of the organisms. By the use of this technic, therefore, it may be possible to determine whether the source from which a strain of streptococci has been isolated is the single factor which determines the results produced by injection of the strain into rabbits or whether, in order to produce these results, the cataphoretic mobility of the organisms must be of a specific value.

Each animal in these experiments received an intravenous injection of 7.5 cc. of a subculture selected from one of the tubes of a series exposed to the high frequency field. Cultures in "glucose-brain broth" and blood agar platings were made from the blood, brain, joints, and other tissues of the animals within 30 hours of the time of injection. In the experiments presented, pure cultures of streptococci, usually either green-producing or indifferent on plates, were obtained from one or more of the tissues of each animal. After 24 hours of incubation at 37°C., 2 cc. of such a culture in glucose-brain broth was centrifuged at 1400 r.p.m. for 10 minutes; it was then drained and the residue was suspended in distilled water. This suspension was used to obtain the cataphoretic velocity distribution of the organisms.

The results of the cataphoretic measurements have been presented as distributions of cataphoretic time, which is related to cataphoretic velocity by the equation:

$$u = \frac{d}{t_c \times (v/1)} = \frac{48.1}{t_c \times 7.0} = \frac{6.9}{t_c}$$

in which t_c is the cataphoretic time and u is the cataphoretic mobility.

The investigations of Rosenow[2, 3, 4] have indicated a relationship

[1] Sheard, Charles, and Pratt, C. B., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 899.

[2] Rosenow, E. C., *Arch. Int. Med.*, 1933, **51**, 327.

[3] Rosenow, E. C., and Jensen, L. B., *Proc. Staff Meetings of Mayo Clinic*, 1930, **5**, 49.

[4] Rosenow, E. C., and Jensen, L. B., *J. Infect. Dis.*, 1933, **52**, 167

between the cataphoretic velocities of streptococci and certain types of disease among patients from whom the organisms have been isolated. Evidence has been presented by him that streptococci obtained from the nasopharyngeal regions of patients with arthritis exhibit a cataphoretic mobility of 2.25 $\frac{\mu/\text{sec.}}{\text{v/cm.}}$ (microns per second divided by volts per centimeter) and that streptococci from nasopharynges of patients with encephalitis and with poliomyelitis show a cataphoretic mobility of either 1.7 or 3.4 $\frac{\mu/\text{sec.}}{\text{v/1}}$. Strains of streptococci isolated in cases of arthritis and showing a characteristic cataphoretic mobility of 2.25 $\frac{\mu/\text{sec.}}{\text{v/1}}$ have been designated by Rosenow as "arthrotropic" because he states that, after intravenous injection of such strains into animals, it was possible, in a majority of instances, to isolate streptococci from the joints of animals and that the organisms so isolated showed the characteristic cataphoretic mobility of the injected strains. Similarly, strains of streptococci isolated in cases of disease of the nervous system, such as encephalitis and poliomyelitis, which show a characteristic cataphoretic mobility of either 1.7 or 3.4 $\frac{\mu/\text{sec.}}{\text{v/1}}$, have been designated by Rosenow as "neurotropic" because streptococci could be isolated from the brain or spinal fluid of animals into which such strains had been injected and the cataphoretic mobility of the recovered organisms was found to be either 1.7 or 3.4 $\frac{\mu/\text{sec.}}{\text{v/1}}$.

In Fig. 1 are shown the results of intravenous injection of control and treated samples of culture No. 5596 (obtained originally from the nasopharynx of a patient with acute epidemic poliomyelitis, the strain having been passed 4 times through animal hosts, producing paralysis in each passage). The control sample of streptococci, having a distribution of cataphoretic time which indicated a majority of organisms at 2 seconds (a mobility of 3.4 $\frac{\mu/\text{sec.}}{\text{v/cm.}}$) was injected into rabbit No. 5969. Streptococci were recovered only from the spinal fluid of this animal, and the cataphoretic distribution of these recovered organisms was identical with that of the injected culture. Subcultures of the 2 samples of this strain which were exposed to the short wave electric field for 12½ minutes, and which showed a cataphoretic distribution indicating an approximately equal division of organisms between the times of 2 seconds and 3 seconds (mobilities of 3.4 and 2.25 $\frac{\mu/\text{sec.}}{\text{v/1}}$), were injected into rabbits Nos. 5970 and 5971. No growth was obtained from the blood of rabbit

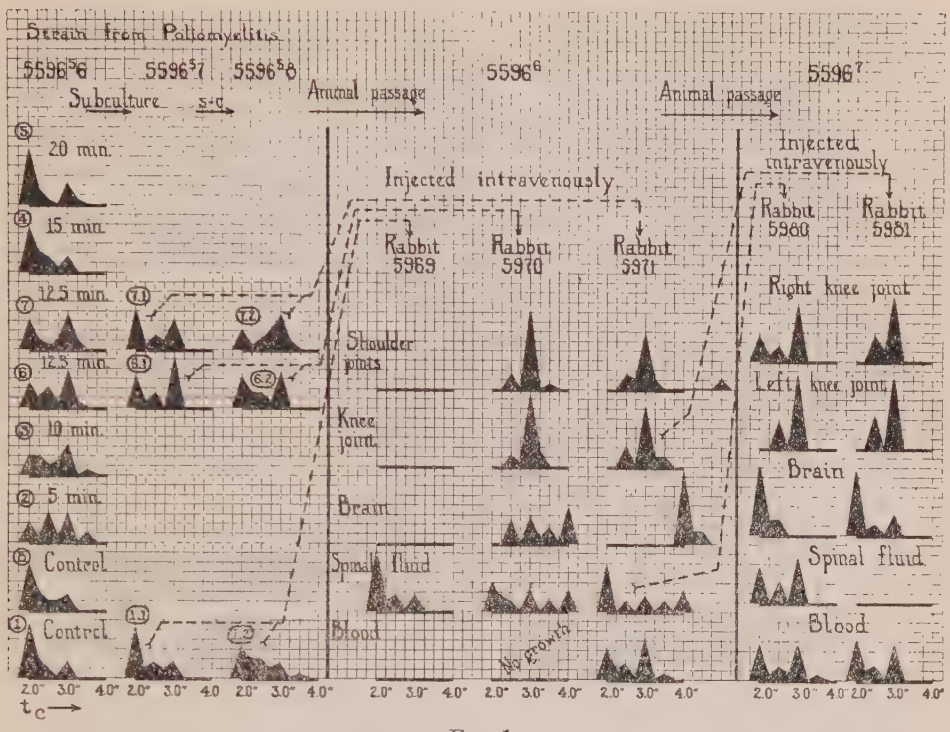

FIG. 1.

Distribution curves of cataphoretic time, showing, left, changes produced by a high frequency field in samples of culture number 5596 and their maintenance in subcultures; middle, distributions obtained from various tissues of rabbits into which the samples of treated culture have been injected intravenously, and right, distributions obtained from various tissues of rabbits into which cultures isolated from rabbit No. 5971 have been injected intravenously.

No. 5970, but the organisms obtained from the cardiac blood of rabbit No. 5971 gave a cataphoretic distribution identical with that of the injected sample of culture. The streptococci recovered from the spinal fluid of both animals showed a distribution spread between 2 and 4 seconds; the major portion of the bacteria, however, were situated near 2 seconds. The organisms obtained from the brain of rabbit No. 5970 showed a spread of distribution from 2.5 to 4 seconds, with slight predominance at the latter value, and those from the brain of rabbit No. 5971 were found to be (practically) entirely at a value of 4 seconds (mobility of 1.7 $\frac{\mu/\text{sec.}}{\text{v/cm.}}$). The cataphoretic distribution of the streptococci obtained from 2 regions in the joints of both animals possessed very marked maxima at 3 seconds (mobility of 2.25 $\frac{\mu/\text{sec.}}{\text{v/1}}$).

Further animal passages were accomplished with 2 of the strains

freshly isolated from rabbit No. 5971, one group of organisms from the spinal fluid having a cataphoretic maximum at 2 seconds and the other, from the knee joint, showing a predominance of organisms at 3 seconds. In the case of the animals Nos. 5980 and 5981, which received these apparently very dissimilar cultures of streptococci, the organisms recovered from respective samples of the blood showed a cataphoretic distribution indicating equal division of the bacteria between the times of 2 seconds and 3 seconds. Organisms were found in the spinal fluid of only one of the animals and the cataphoretic distribution of these was similar to that of the bacteria from the blood. From the brains of both animals streptococci were obtained which showed a marked cataphoretic predominance at 2 seconds. From the 2 knee joints of both animals streptococci were removed, and the cataphoretic distributions were uniformly concentrated at 3 seconds (mobility of 2.25 $\frac{\mu/\text{sec.}}{\text{v/1}}$).

Additional investigations concerning certain effects of the injection of streptococci into rabbits will be presented elsewhere.[5]

Conclusions. 1. Strains of streptococci, in which alterations in cataphoretic velocities have been produced by exposure to a high frequency field (wavelength of 11 meters), when subsequently injected into rabbits are found as frequently in certain tissues as are the control (unexposed) strains. 2. Irrespective of the type of cataphoretic distribution of the injected strain, the streptococci which are isolated from brain tissue exhibit a type of cataphoretic velocity distinctly different from the organisms which are isolated from tissues of the joints.

7904 C

Platelet and Blood-Cell Counts in Newborn During First Two Days of Life.

DAVID ROSENBLOOM. (Introduced by F. H. Falls.)

From the Department of Obstetrics and Gynecology, University of Illinois, Chicago.

The purpose of this study was to establish a set of blood-platelet-count standards in infants, based upon an accurate method of counting. Red cell counts, white cell counts, and hemoglobin determina-

[5] Pratt, C. B., Sheard, Charles, and Rosenow, E. C., *Protoplasma*, 1935.

tions were made concomitantly. McLean and Caffey,[1] Lippman,[2] Jarcho,[3] Slawik,[4] Keilmann,[5] Lucas,[6] and Farnow[7] have furnished various standards.

The counts are made with a diluting fluid consisting of 100 cc. of a 3.8% aqueous solution of sodium citrate, 0.2 cc. of 40% formaldehyde, and 0.1 gm. of brilliant cresyl blue, kept ice-cold. A red-count pipette is filled to the 1.0 mark with diluting fluid, it is rapidly inserted into the drop of blood, and blood is drawn into the pipette approximately to the 0.5 mark, and diluting fluid is then immediately drawn in to the 101 mark. The pipette is then shaken. With a second pipette, an accurate red-cell count is made, using Hayem's solution. The hemocytometer is charged with the contents of the first pipette, the platelets are counted (in the same manner as red cells are counted), and then in the same fields the red cells are counted. The hemocytometer is then charged with the contents of the second pipette and a count is made. The latter number is an accurate absolute count of the red cells. The following proportion gives the number of blood platelets:

$$\frac{\text{Red cells (Pipette 1)}}{\text{Platelets (Pipette 1)}} :: \frac{\text{Red cells (Pipette 2)}}{X}$$

At the same time that the blood was taken from each infant for the platelet counts (through a deep heel puncture), a white-cell count was made and hemoglobin was read with a Dare hemoglobinometer (measuring 13.88 gm. per 100 cc. as 100%).

In all, 108 full-term infants were studied. Care was taken to exclude those babies who appeared abnormal in any way, those who developed icterus neonatorum within the first or second days of life,

TABLE I.

Cases studied	Age, hr.	Platelets	White Blood Corpuscles	Red Blood Corpuscles	Hemoglobin %
30	0-4	310,000	20,900	6,480,000	120.9
22	5-8	351,000	23,390	6,770,000	119.0
21	9-12	471,000	24,800	6,349,000	116.6
24	13-24	280,000	24,200	6,169,000	113.8
10	25-48	387,000	19,720	6,450,000	108.6

N.B. These figures are averages.

1 McLean and Caffey, *Am. J. Dis. Chil.*, 1925, **30**, 810.
2 Lippman, *Am. J. Dis. Chil.*, 1924, **27**, 473.
3 Jarcho, *Arch. Ped.*, 1930, **47**, 230.
4 Slawik, *Z. f. Kinderheilkunde*, 1920, **25**, 212.
5 Keilmann, *Monatschr. f. Kinderheilkunde*, 1922, **23**, 323.
6 Lucas, *Am. J. Dis. Chil.*, 1921, **22**, 525.
7 Farnow, *Jahrb. f. Kinderheilkunde*, 1926, **112**, 47.

and those who presented signs of dehydration. The results are seen in Table I.

7905 C

Cholesterol of Maternal and Fetal Blood at the Conclusion of Pregnancy.

DAVID ROSENBLOOM. (Introduced by F. H. Falls.)

From the Department of Obstetrics and Gynecology, University of Illinois, Chicago.

This paper concerns the relative concentrations of cholesterol in the maternal and in the fetal circulations. By determining the quantity of a certain substance on both sides of the placenta, evidence can be presented concerning the transmission of that substance through the placenta. Fetal blood is not a simple dialysate or filtrate of the maternal blood, but the placenta exerts a specific and definite selective power over certain of the substances which pass into it and which might conceivably enter the fetal circulation.

The amount of cholesterol in human blood is 150-190 mg. per 100 cc.[1, 2] It is increased in the blood of pregnant women.[3–9] Several investigators have studied cholesterol in maternal and in fetal blood.[10–13]

The modified method of Bloor[14] was used for the cholesterol determinations. Stoppered one-ounce blood bottles containing a small amount of sodium oxalate were included in the sterilizer

1 Hawk and Bergeim, *Practical Physiological Chemistry*, 9th ed., Philadelphia, P. Blakiston and Co., 1926, p. 357.

2 Mathews, *Physiological Chemistry*, 4th ed., New York, Wm. Wood and Co., 1927, p. 29.

3 Barsony, *Zentralbl. f. Gynak.*, 1930, **54**, 1811.

4 Hellmuth, *Zentralbl. f. Gynak.*, 1931, **51**, 802.

5 Strauss and Schubardt, *Zentralbl. f. inn. Med.*, 1922, **43**, 425.

6 Rosen and Krasnow, *Am. J. Obs. Gyne.*, 1927, **14**, 321.

7 Chauffard, Laroche, and Grigaud, *Obstetrique*, Paris, 1911, **4**, 481.

8 Benda, *Arch. f. Gynak.*, 1923, **116**, 508.

9 Fluhmann, *Am. J. Obs. Gyne.*, 1926, **12**, 774.

10 Slemons and Stander, *Bull. Johns Hop. Hosp.*, 1923, **34**, 7.

11 Slemons, *Bull. Johns Hop. Hosp.*, 1916, **27**, 343.

12 Slemons and Stander, *Trans. Am. Soc. for Advancement of Clin. Invest.*, 1918.

13 Slemons and Curtis, *Am. J. Obstetrics*, 1917, **75**, 569.

14 Bloor, *J. Biol. Chem.*, 1922, **52**, 191.

drums containing the sterile linen and instruments for each obstetrical delivery. Immediately after the birth of the baby the umbilical cord was clamped between hemostats and cut. One of the hemostats was released and 5 cc. of blood were placed in the bottle, which was then stoppered and shaken. As soon as the mother was returned from the delivery room (usually within 15 minutes), 5 cc. of her blood were taken from the median basilic vein by a hypodermic syringe and put into an oxalate blood bottle. The cholesterol determinations were run simultaneously on both infant and maternal bloods. By carrying out the procedures in the manner described, the blood specimens represented the maternal and fetal bloods at the very end of pregnancy.

In all, 65 cases were studied (by "case" is meant a cholesterol determination on both maternal and fetal blood). Care was taken to include in the series only normal, full term pregnancies, with normal, healthy infants. No operative cases were included. In all of the cases, light obstetrical ether anesthesia was used at the end of the second stage of labor, and deep narcosis was never necessary. At the time the maternal blood samples were drawn, the patients were already conscious.

TABLE I.
Maternal Blood Analyses.

Mg. cholesterol per 100 cc.	No. cases in this range
100-125	3
126-150	7
151-175	7
176-200	8
201-225	12
226-250	12
251-275	5
276-300	7
301-325	1
326-350	3

Aver. of 65 cases: 222.7 mg. per 100 cc.

TABLE II.
Infant Blood Analyses.

Mg. cholesterol per 100 cc.	No. cases in this range
50- 75	8
76-100	6
101-125	30
126-150	9
151-175	10
176-200	1
201-225	0
226-250	0
251-275	1

Aver. of 65 cases: 120.4 mg. per 100 cc.

In Table I are listed the results of cholesterol determinations upon the maternal bloods. These have been tabulated, within restricted ranges of cholesterol, in terms of mg. per 100 cc. In Table II are listed in similar manner the results obtained from determinations upon the infant bloods. The average of the 65 cases for the maternal blood is 222.7 mg. per 100 cc. For fetal blood, the average is 120.4 mg. per 100 cc.

In conclusion it can therefore be said that there is a far higher cholesterol content in maternal blood than in fetal blood at the conclusion of pregnancy. Even if the placenta transmits substances by simple diffusion,[15, 16, 17] it must also have a selective control over the passage of cholesterol from mother to fetus, or if the placenta is impermeable to cholesterol, it must be assumed that the fetus is able to synthesize in its own body a considerable amount of cholesterol.

7906 C

Duration of Estrus in Ovariectomized and Adrenal-Ovariectomized Rats Before and After Theelin.

FREDERICK E. EMERY AND EDWARD L. SCHWABE.

From the Department of Physiology, University of Buffalo.

Within the last few years numerous papers have appeared on several phases of the adrenal problem without adding, as Rogoff[1] has recently discussed, any very definite contribution. The literature on the sex-relationship has been especially confusing; and the many references cited by Kroc and Martin[2] indicate that total adrenalectomy may cause no change, a slight modification or complete inhibition of the estrus cycles. These authors made a point of the fact that the weight-loss of adrenalectomized rats must be restored before normal estrus is again established following injections of the cortical hormone. Yet this same result, as to normal estrus in adrenalectomized rats, may be obtained by allowing the animals to drink salt solutions (Kutz, *et al.*[3]).

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15 Schlossman, *Der Stoffaustausch zwischen Mutter und Frucht durch die Placenta*, Munich, J. F. Bergmann, 1933.

16 Sinclair, *Am. J. Physiol.*, 1933, **103**, 73.

17 Masciotta and de Hoz, *Presse Med.*, 1933, **41**, 293.

1 Rogoff, J. M., *J. Am. Med. Assn.*, 1934, **103**, 1764.

2 Kroc, R. L., and Martin, S. J., *Am. J. Physiol.*, 1934, **108**, 438.

3 Kutz, R. L., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 331.

This led us to study, by a different procedure, the theelin-inhibition problem previously reported.[4] The present approach has been first, to compare ovariectomized and adrenal-ovariectomized rats as to the estrus occurring on successive days following the operation; and second, the duration and reoccurrence of estrus that followed various doses of theelin. The estrus cycles were taken daily, by the pipette method, for 2 weeks or longer preceding and following the operation. Six to 10 days following the operation, all rats were placed on Ringer's solution as their only source of drinking water. A few animals died before the sixth day; but these are not considered in the data. All animals were autopsied. In 4 of the 60 rats composing the adrenal-ovariectomized group, adrenal fragments were found. Ovarian fragments were not seen in either group.

The per cent of rats in estrus each day for 10 days before and 12 days after ovariectomy (group O) is shown in Fig. 1; and similar results for adrenal-ovariectomized rats (group A-O) are found in Fig. 2. The 2 figures are almost identical in every way. Before operation, the per cent for group O averaged 45.1%, and for the A-O group 42.8%. These figures are slightly higher than the usual mean for our colony, but within the range of previous results.[4] The phase of the cycle at the time of operation seemed to have no bearing upon the post-operative effects.

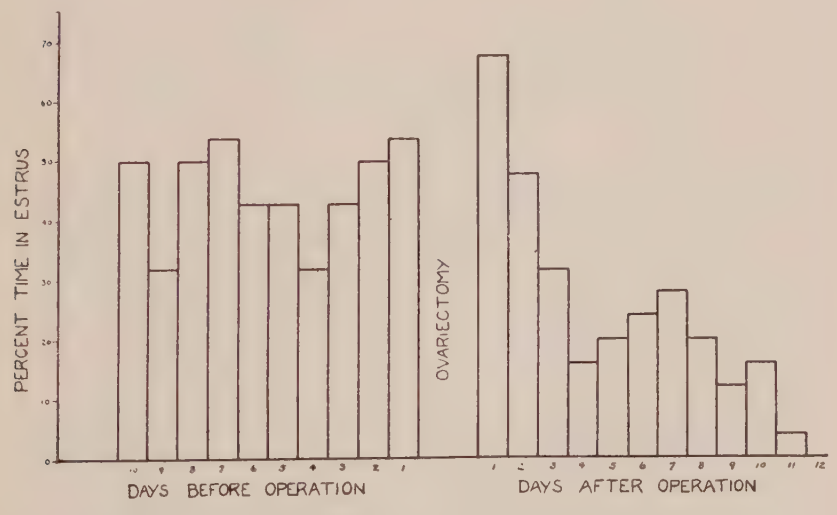

FIG. 1—Group O.
The per cent of rats in estrus each day for 10 days before and 12 days after ovariectomy. Data from 25 rats.

4 Emery, F. E., *Anat. Rec.*, 1933, **57**, 315.

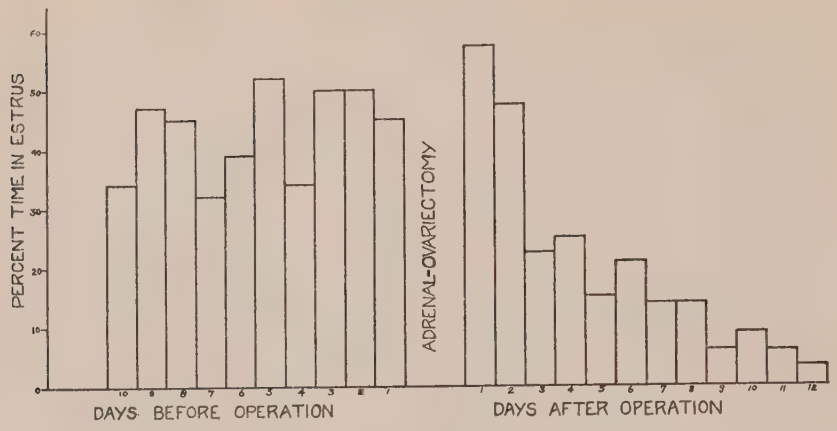

FIG. 2—Group A-O.
The per cent of rats in estrus each day for 10 days before and 12 days after adrenal-ovariectomy. Data from 40 rats.

It is interesting to note that the day following the operation more rats were in heat than on any other day. We were surprised to find so many estrus periods during the first 8 post-operative days in both groups; and especially, to note that the number in estrus on the sixth and seventh days was equal to or greater than those of the third day (Figs. 1 and 2). Even 12 to 15 days after total ovariectomy, estrus may occur. The intensity of the reaction is greatly diminished after the third day; and a vaginal smear of ++++ is seldom seen. We believe these results can be interpreted as estrous occurring on a lower level; but still maintaining some rhythm for 10 days or more after ovariectomy. Similar phenomena have been reported in the spontaneous activity of rats after ovariectomy.[5]

TABLE I.
Number of Rats in Estrus and Duration of Estrus in Days after Various Doses of Theelin in Ovariectomized and Adrenal-ovariectomized Animals.

	Theelin ½ R.U.			Theelin 1 to 5 R.U.			Theelin 6 to 10 R.U.		
Group	No. of rats	No. in estrus	Estrus reoccurred	No. of rats	Duration of estrus days	Estrus reoccurred	No. of rats	Duration of estrus days	Estrus reoccurred
A-O	16	4	none	19	2.1	4	12	2.2	3
O	16	3	"	19	1.8	2	24	2.3	5

A study of the data on theelin injections (Table I) shows that here again both groups reacted in a similar way. To facilitate the comparison, we have put the same number of rats in both groups, excepting the large dose of theelin, where a total of 12 and 24 are

[5] Hemmingsen, Axel M., *Skand. Arch. f. Physiol.*, 1933, **65**, 97.

considered. It will be seen that sub-minimal doses of theelin caused a reaction in 3 cases in group O and 4 cases in the A-O group. The duration of the estrus is the same in the 2 groups; as is also the number of cases where estrus reoccurred a few days after the first estrus.

Another series of 30 rats ovariectomized at weaning were divided 8 weeks later into 2 groups of 15 rats each. Bilateral adrenalectomy was performed on one group, and bilateral laparotomy on the other or control group. On the 2 days following operation, theelin was injected in 4 equal doses to a total of 2 R.U. In the adrenalectomized group 13 animals came into estrus, as compared to 11 in the control group. Thus the larger doses of theelin that are required to bring about estrus in ovariectomized rats of long standing are not reduced by adrenalectomy.

Summary. Total adrenalectomy in rats has little or no effect on (a) the estrus cycles that occur after ovariectomy; (b) reducing the minimal dose of theelin; (c) the duration of estrus following 1 to 10 R.U. of theelin; (d) the tendency for estrus to reoccur after a positive reaction to theelin; and (e) reducing the dose of theelin needed to produce estrus in ovariectomized rats of long standing.

7907 P

Depressor Action of Extracts of Burned Skin.

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Extracts were made of normal and burned skin of rabbits by the method of Chang and Gaddum.[1] The skin was burned at temperatures varying from 70°C. to 210°C. and removed from 3 minutes to 48 hours after burning. The extracts were then tested by their action after intravenous injection on the blood pressure of 7 rabbits and 10 cats.

Results. Extracts of burned skin of 10 anesthetized rabbits caused an immediate, but transient, fall in blood pressure. The curve was similar to that following acetylcholine, but the recovery was slower

*Carnegie Research Fellow.

1 Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

and was not always followed by a rise above the original level. Recovery was slower in the rabbit than in the cat. The depressor action was not affected by the injection of atropine (0.1 mg. per kg.), and was not reduced by treating the extract with alcohol to precipitate adenosine and its derivatives; and since depression of the blood pressure of rabbits was obtained, the depressor substance was not mainly histamine. Later experiments showed that when the extract was boiled with alkali and then neutralized, the depressor action did not disappear. The substance mainly responsible for the action was therefore not the "P" substance of Euler and Gaddum.[2] The depressor substance has not been identified. Extracts of normal skin of 5 rabbits were qualitatively similar to those of burned skin in depressor action.

Quantitative Comparisons. The depressor action of the extracts was compared with that produced by a standard solution of acetylcholine and roughly expressed in γ of acetylcholine equivalent per gm. of tissue. The extracts are named according to the time after burning or removal of tissue and to the temperature at which the heat had been applied. The 5½ hour, 24 hour, and 48 hour control extracts contained roughly 2.5 γ ac.-equiv. The 3 minute 70°, 3 minute 210°, and 48 hour extracts averaged a like amount, whereas the extracts taken during the intervening period, including the 3 hour 110°, 4 hour 105°, 24 hour 170°, and 24 hour 110° extracts were not so active, containing usually less than 1 γ ac.-equiv. At the 3 minute and 48 hour periods the subcutaneous edema fluid was small in amount as in normal skin, whereas at the intervening times, considerable edema fluid was present. This suggests that the depressor content of skin is not significantly altered by the application of heat, except insofar as the depressor substances become diluted by edema fluid.

Summary. (1) Trichloracetic acid extracts of the normal skin of rabbits have a depressor activity which is not due to acetylcholine, adenosine, histamine, or the "P" substance of Euler and Gaddum. (2) Extracts of rabbits' skin to which heat had been briefly applied at intervals of from 3 minutes to 48 hours previously, contain an apparently identical depressor substance. Such extracts show no increased depressor activity, but sometimes a diminution, which is probably due to dilution of the depressor content of normal skin by edema fluid.

[2] Euler, U. S., and Gaddum, J. H., *J. Physiol.*, 1931, **72**, 74.

7908 C

Attempts to Produce Immunity with Large Quantities of Killed Herpes Virus.

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It has long been agreed and repeatedly stated that one of the principal differences between viruses and bacteria is the fact that apparently no immunity can be produced with a virus which is not living. So far there has not been a single acceptable experiment which would indicate that immunity may be produced with any virus which has been definitely destroyed by heat, chemicals, or any other means. That quite the opposite is true, with at least many bacterial forms, is well known. However, though the immunology of viruses has been studied a great deal, there have been no experiments reported, so far as we are aware, in which attempts to produce immunity with killed virus in very large quantities have definitely ruled out this possibility. It is obvious that if immunity with any virus depends upon quantity of the antigen employed and not upon the living nature of the virus much might be accomplished, with greater safety, by simply concentrating the virus for immunization purposes.

We have sought to test, by critical experiment, this possibility and have employed the L. F. strain of herpes virus for our experiments. Our herpes strain is one which one of us (E. B. McK.) brought to this country from Brussels, Belgium, in 1925, and for nearly 10 years it has been propagated in the brains of rabbits in this country. It regularly kills rabbits within 3 to 5 days when given in doses of 0.25 cc. of a 10% suspension of infected rabbit brain by the intraspinous route. For our experiment we selected 3 potent, whole, recently herpes-infected rabbit brains and emulsified these brains in 75 cc. of saline. The virus suspension was then heated at between 65 and 68.5°C. in a water bath for one hour. The heated virus suspension was then tested intraspinously in 2 normal rabbits with 0.5 cc. each. Both of these animals survived showing conclusively, considering the probable amount of virus which would have been present had it not been destroyed, that the herpes virus had been killed at this temperature for the length of time indicated.

After testing for the viability of the virus, and finding that the virus had been destroyed, a large healthy rabbit was injected sub-

cutaneously in various localities with the balance of the killed virus suspension, *i. e.*, 73 cc. which contained practically the entire 3 brains originally emulsified. Small amounts of the virus suspension were employed for aerobic culture purposes and these tests indicated that no viable bacterial forms (aerobic) were present in the emulsion.

After vaccination with this enormous quantity of killed virus the animal was allowed to remain in his cage for one month, when he was tested intraspinously with 0.5 cc. of fresh herpes brain emulsion. After 3 days the animal died with typical symptoms of herpes encephalitis. The brain was subsequently found to contain potent herpes virus when tested in other animals.

This experiment is an attempt to show conclusively that with killed herpes virus no immunity can be produced in rabbits even when *enormous quantities* of killed virus are employed for this purpose. It is likely that the same pertains to other viruses and gives additional evidence to support the principle, long held, that *living virus is essential* in the production of virus immunity.

7909 P

Immunological Studies with Poliomyelitis and Vaccine Viruses in Monkeys.

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Recent work on attempts to produce active immunity in monkeys with poliomyelitis virus by McKinley and Larson[1] and quite recently by Kramer, Schaeffer and Park,[2] Brodie,[3, 4] and Kolmer and Rule[5, 6] has indicated that it is possible to produce such immunity with virus treated with sodium ricinoleate, immune serum or formalin. In addition Park and his group and Kolmer have em-

[1] McKinley, J. C. and Larson, W. P., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 297.

[2] Kramer, S. D., Schaeffer, M., and Park, W. H., *J. Immunol.*, 1934, **27**, 199.

[3] Brodie, M., *Science*, 1934, **79**, 594.

[4] Brodie, M., *J. Immunol.*, 1935, **28**, 1.

[5] Kolmer, J. A. (with the assistance of A. M. Rule), *Am. J. Med. Sci.*, 1934, **188**, 510.

[6] Kolmer, J. A., and Rule, A. M., *J. Immunol.*, 1934, **26**, 505.

ployed such vaccines without harm in children and adults. Aycock and others have given live virus to monkeys subcutaneously without producing paralysis. Brodie[7] has demonstrated that a single subinfective intracutaneous inoculation of active virus gives rise to an immunity which appears at or soon after the sixth day and reaches its height by the twentieth day. The immunity so produced persists for at least one year. A second injection of the antigen gives rise to considerable additional immunity. These various observations give hope that some method may be found to produce immunity against this disease.

We here report briefly a method we have been studying for producing immunity with poliomyelitis virus which involves the use of minute amounts of living and untreated virus given intracutaneously to monkeys. Such a method in order to be of practical use should be harmless and it should produce a degree of immunity sufficient to protect the animals against a subsequent paralyzing dose of poliomyelitis virus given intracerebrally. While the various methods mentioned above depend upon treatment of the virus with some substance such as formalin or a fatty acid salt it may be of practical significance to determine if live, untreated, virus in small doses (similar to vaccine virus) may be employed with efficiency and without danger. Without doubt the chemically treated virus mentioned above is still living for there is no evidence that killed virus of any type will produce immunity. Since viruses are notoriously intracellular parasites it would seem that the chemical treatment of a virus suspension would first destroy extracellular virus and the intracellular virus would then be released in the body tissues very slowly by destructive action upon the virus-containing cells. Herein may be the explanation for the success of such chemically treated virus vaccines.

To approach the problem from another angle we decided to study methods of producing immunity with poliomyelitis virus with live, untreated, virus. We have found that when we employ minute doses, such as 0.5 cc. of a 16% emulsion of our polio infected monkey cord (Aycock strain), and introduce the virus intracutaneously we cannot protect the animal against our virus given, after 6 weeks, intracerebrally. Such animals develop paralysis. However, when we mix such small doses of live, untreated, virus of such potency with the virus of vaccinia and vaccinate the animals by intracutaneous injection we find that such a method may produce a solid immunity in monkeys so that, 6 weeks following vaccination,

[7] Brodie, M., *J. Immunol.*, 1934, **27**, 395.

they are fully protected against intracerebral injections of paralyzing doses of live virus.

Thomsen[8] reported briefly on a similar experiment in 1912 but in the experience of this author no immunity was produced in the small number of animals employed in the course of his work. However, this work was reported shortly after the virus of poliomyelitis was discovered. We have not determined the mechanism involved in this reaction but we have postulated the possibility of the gradual entrance of poliomyelitis virus into the tissues of the animal through the aid of the concomitant virus infection, vaccine virus, which may open the pathway for the poliomyelitis virus. In effect we may have a situation similar to chemically treated virus, if our hypothesis is correct, where the living virus is slowly absorbed and in insufficient dosage to produce paralysis. It would be interesting to know if, after long residence in the monkey, the virus of poliomyelitis has undergone some change biologically and, if returned to man, will no longer produce paralysis when given subcutaneously or intracutaneously as we have described.

7910 C

Effect of Age of a Specific Medium on Morphology of Colonies of Certain Pathogenic Fungi.*

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In this work batches of media were allowed to age at laboratory temperature, planted with organisms and morphological differences observed. In aging, the medium loses water and the nutrient concentrates so that while the ratio of ingredients is probably approximately the same the moisture content, as will be noted below, becomes much less. Our routine W medium, consisting of 4% peptone, 1% dextrose, 1½% agar and adjusted to pH 5.6, was used. The age of media which was placed on laboratory bench tops varied by approximately 10-day intervals from fresh medium to 90-day-old medium.

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[8] Thomsen, O., *Z. f. Immunitäts.*, 1912, **14**, 198.

* Contribution No. 50 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

The following representative fungi and 2 non-pathogenic saprophytes, *Lichtheimia sp.* and *Scopulariopsis brevicaulis,* were studied: *Achorion schoenleinii, Acladium castellani, Candida candida, Endodermophyton indicum* (Castellani), *Endodermophyton tropicale, Endomyces capsulatus, Endomyces dermatitidus, Epidermophyton cruris, Epidermophyton inguinale, Epidermophyton rubrum* (Castellani), *Glenospora gammeli, Geotrichum bachmann, Indiella americana, Microsporon audouini, Microsporon felineum, Microsporon gypseum, Monosporum apiospermum, Monilia albicans, Oöspora humi, Sporotrichum schenkii, Trichophyton balcaneum* (Castellani), *Trichophyton crateriforme, Trichophyton decalvans* (Castellani), *Trichophyton granulosum, Trichophyton gypseum, Trichophyton gypseum* asteroides, *Trichophyton gypseum* lacticolor, *Trichophyton interdigitale, Trichophyton japonicum, Trichophyton louisiana* (Castellani), *Trichophyton niveum, Trichophyton purpureum, Trichophyton sulfureum, Willia anomala.*

With age there was retraction and contraction of the medium. This was accompanied by less space for the growth to spread over, with some resultant lessening of growth. In 13-weeks-old medium there was enough shrinkage so that after a month of growth only a dry scale remained. Yet growth occurred in all instances with the exception of *Trichophyton louisiana.* Thirteen-week growths were smaller, much dried yet retained in most instances some of the typical morphology seen on fresh medium. In many instances the growths on 11- and 12-week media were much dried but showed good differentiation. The growths on younger media were good and showed morphology in most instances as good as that on fresh medium. *Trichophyton japonicum* showed more coloring of the mycelia on the older media but most of the other growths showed a color variation which could not be ascribed to age. *Microsporon gypseum* showed a tendency to lose its powder in old media and develop low white aerial mycelium while old media growths of *Epidermophyton inguinale* seemed to show more pigment. The finely waxy radiate border of *Trichophyton purpureum* and the waxy ribbed border of *Trichophyton rubrum* became somewhat less distinct on old media but retained in most instances their yellow color in the early period of growth. In growths which showed frosting on fresh medium there was a tendency on old media to show decrease in this appearance and increase in white mycelium. In the very old media *Microsporon audouini* showed less of the soft mycelium and more the coarser of deeper tan color.

The maintenance of morphology in old media was striking. This

is the converse of the decided effect on morphology of change of the ratio of dextrose to peptone which has been very evident in our studies. Possibly the maintenance of the same ratio of ingredients is the important factor. The reaction which was adjusted to pH 5.6 became as a rule slightly more acid but did not fall below 5.2. In other studies of W medium in which the pH had been varied from 5.2 to 8.0, we found no marked morphological variations. It is logical therefore that this pH change would not be responsible for variation.

In order to visualize the loss of weight we have weighed tubes of W medium over periods of time, each tube containing 10 cc. of medium of a weight of approximately 9.513. Over the period of the study it was found that the medium lost on an average 8.6 mg. per gram in weight per day. For the 90-day period this meant a loss of 7.363 gm. of water. This left a mass of 2.150 gm. in each tube of which 0.65 gm. were the solids added in the form of dextrose, peptone and agar, and 2.085 moisture. This meant a concentration of ingredients of almost 4½ times. In other words, in the oldest medium we were planting our organisms on medium of approximate concentrations of agar of 6.75%, of peptone of 18%, and of dextrose of 4.5%. Nevertheless, the organisms maintained an approximately individually similar morphology as long as this ratio was maintained. This loss of moisture was somewhat less the first few weeks. It is possible that retraction of the medium from the glass and splitting with increased surface accounted for some of the increase. The latter weighings also showed a tendency to slight decrease in weight loss.

This work emphasizes the length of time, namely, approximately 10 weeks, that this medium can be allowed to stand at laboratory temperature and still show good growth with good differentiation. It also suggests the possibility that maintenance of similar peptone-dextrose ratios may mean similar gross colony morphology. The weight loss, which varies with temperature and humidity and which averaged approximately 8.6 mg. per gram of substance per day, was greater than weight loss obtained in the summer with windows open and no artificial heat, which averaged 7 mg. per gram of substance per day. This was due undoubtedly to a higher laboratory temperature and a drier atmosphere in the artificially heated laboratory.

7911 C

Glycolysis in the Blood of the Goat.*

JESSIE T. CUTLER. (Introduced by P. D. Lamson.)

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A previous communication[6] reported that the normal blood sugar of the goat ranges from 24 to 65 mg. per 100 cc., levels considerably lower than those reported for other laboratory animals. A second difference between the blood of the goat and that of other animals is indicated by the data given below, namely, a difference in the behavior of the inorganic phosphate during glycolysis.

It has been repeatedly observed [2, 7, 10, 15] that the fall in blood sugar which occurs in shed blood is accompanied by typical changes in the level of the inorganic phosphate. During the first few hours of glycolysis, while the sugar is being destroyed rapidly, the inorganic phosphate decreases. When the sugar has practically all disappeared and glycolysis has, perforce, either stopped or slowed to a very low rate, there is a rapid and extensive increase in the level of the inorganic phosphate. These changes during glycolysis have been observed in the blood of the dog, the rabbit and the human.

Data are given below on the changes in sugar, lactic acid, and inorganic phosphate which occur in the blood of the goat, when defibrinated and incubated at body temperature. The blood of dogs was used for control purposes.

For these studies, 75 to 100 cc. of blood was drawn from the jugular vein and defibrinated with a glass rod. Samples were withdrawn immediately for the determination of sugar, lactic acid, and inorganic phosphate. The flask containing the remainder of the blood was stoppered and placed in the incubator at a temperature of 37° to 38°C. Aseptic precautions were not taken as it has been shown that slight bacterial contamination alters neither the glycolytic rate[8, 13] nor the course of the accompanying changes in the inor-

*This investigation was aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

6 Cutler, J. T., *J. Biol. Chem.*, 1934, **106**, 653.

2 Barrenschien, H. K., and Hübner, K., *Biochem. Z.*, 1930, **229**, 329.

7 Engelhardt, W. A., and Braunstein, A. E., *Biochem. Z.*, 1928, **201**, 48.

10 Guest, G. M., *J. Clin. Invest.*, 1932, **11**, 555.

15 Roche, A., and Roche, J., *Bul. de la Soc. Chim. Biol.*, 1929, **11**, 549.

8 Falcon-Lesses, M., *Arch. Int. Med.*, 1927, **39**, 412.

13 Mackensie, G. M., *J. Exp. Med.*, 1915, **22**, 757.

ganic phosphate.[10] Samples were withdrawn for the determinations at intervals of 1 to 4 hours, and the experiments were continued for 12 to 24 hours.

The sugar was determined by Benedict's method,[4] the lactic acid by the method of Friedmann and Kendall,[9] and the inorganic phosphate by Briggs modification[5] of the method of Bell and Doisy.[3] All determinations were made on whole blood.

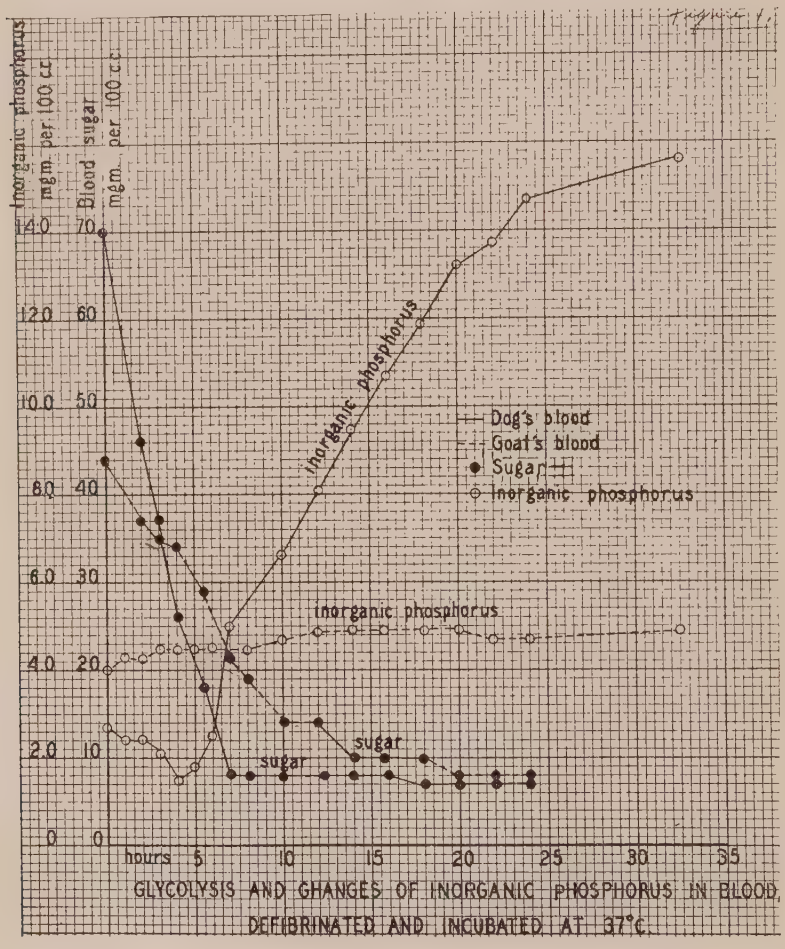

FIG. 1.

Glycolysis and accompanying changes in inorganic phosphate in blood of the dog and of the goat.

4 Benedict, S. R., *J. Biol. Chem.*, 1926, **68**, 759.

9 Friedmann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, **82**, 23.

5 Briggs, A. P., *J. Biol. Chem.*, 1922, **53**, 13.

3 Bell, R. D., and Doisy, E. A., *J. Biol. Chem.*, 1920, **44**, 55.

Sugar disappears progressively from the blood of the goat as it does from that of other animals. The glycolytic rate is, however, considerably slower in goat blood than in dog blood. In our experiments the rate of fall of sugar in the blood of goats varied from 2.2 mg. to 6.2 mg. per 100 cc. per hour, with an average of 4.1 mg., while in dog blood, the rates ranged from 7.0 mg. to 9.2 mg. per 100 cc. per hour, with an average of 8.1 mg. The normal course of glycolysis in the blood of either the goat or the dog may be noted in Fig. 1. The sugar falls progressively over a period of 6 to 14 hours after the blood is drawn until it reaches a level of 6 to 8 mg. per 100 cc., at which level it remains for as long as 48 hours. We believe that this residue is not glucose, but some other reducing substance.

As the glucose diminishes, lactic acid appears in the blood of the goat, as in that of other animals, in amounts equal to the sugar which disappears. The figures presented in Table I show that during the first 6 hours of glycolysis, the lactic acid formed and the sugar lost are equal, within the limits of error of the methods used. After 6 to 8 hours the lactic acid apparently begins to be destroyed, since it decreases in amount from this point. Except for the difference in rate, glycolysis in goat blood is like that in dog blood, in that the progressive loss of sugar can be accounted for by a simultaneous appearance of lactic acid. However, a suggestion of a divergence in the intermediate steps in the process in the blood of the 2 species is found in the behavior of the inorganic phosphate during glycolysis.

The changes in the level of the inorganic phosphate during glycolysis in dog blood in our experiments are like those reported by Roche and Roche[15] and those observed in the blood of rabbits and normal humans by Guest.[10] During the first 4 to 6 hours of incubation the concentration of the inorganic phosphate decreases. It then begins to increase rapidly and continues to do so for 10 to 20 hours. (Fig. 1.)

In goat blood these characteristic changes do not occur during glycolysis. (Fig. 1.) During 24 hours of incubation there is only a slow continuous rise in the level of the inorganic phosphate, which amounts to but 1 or 2 mg. per 100 cc. This rise bears no apparent relationship to glycolysis as it continues at approximately the same rate whether sugar is present or has all disappeared.

Further evidence of the usual relationship of inorganic phosphate to glycolysis is furnished by the observation, reported by

TABLE I.
Production of Lactic Acid During Glycolysis, mgm. per 100 cc.

Animal	Blood when drawn		After 2 hours		After 6 hours		After 10 hours		After 14 hours	
	Sugar	Lactic Acid	Sugar	Lactic Acid	Sugar	Lactic Acid	Sugar	Lactic Acid	Sugar	Lactic Acid
Goat 5	49	6	38	16	19	35	13	41	9	41
" 33/3/5	64	19	55	28	39	43	9	52	8	59
" 33/3/6	69	17	58	30	36	51	8	47	8	44
" 33/3/3	52	16	33	36	19	50	8	50	8	47
" 33/3/4	49	14	38	25	19	45	7	46	7	45
" 33/9/1	47	40*	35	56	18	72	8	69	8	64
" 33/3/2	43	13	35	20	20	34	9	37	8	40
Dog 33/3/1	68	11	43	37	22	58	11	65	10	55

* Animal struggled before sample was drawn.

many,[2, 10–12, 15–17] that processes which accelerate glycolysis result in a greater preliminary fall of the inorganic phosphate. From the processes known to accelerate glycolysis, the addition of glucose to the blood was selected for application to goat blood.

Our results on dog blood were entirely similar to those reported in the literature. However, the addition of glucose to goat blood (50 to 100 mg. per 100 cc.) never resulted in a fall of the inorganic phosphate. The usual result was a slight but definite delay in the gradual rise which occurs in the untreated blood. (Fig. 2.) Such a

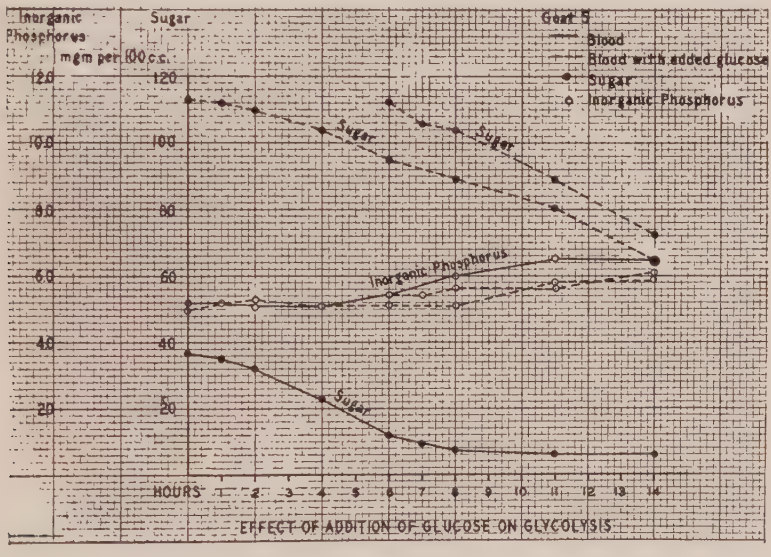

FIG. 2.

Effect of addition of glucose on glycolysis and changes in inorganic phosphate in goat blood. Glucose was added to one portion of the sample at beginning of experiment. Control sample was divided after 6 hours of incubation and glucose added to a portion of it at that point.

delay was produced whether glucose was added immediately after the blood was drawn, or at a time several hours later when the original sugar had almost disappeared. It should be noted, also, that the addition of glucose to goat blood never produced more than a very slight acceleration of the glycolytic rate. In about half the cases there was no acceleration whatsoever.

It has been reported also that measures which check or prevent

11 Kawashima, Y., *J. Biochem.*, Japan, 1923, **3**, 273.

12 Lawaczeck, H., *Biochem. Z.*, 1924, **145**, 351.

14 Martland, M., Hansman, F., and Robison, R., *Biochem. J.*, 1924, **18**, 1152.

16 Rona, P., and Doblin, A., *Biochem. Z.*, 1911, **32**, 489.

17 Rona P., and Iwasaki, K., *Biochem. Z.*, 1927, **184**, 318.

glycolysis result in an immediate release of inorganic phosphate.[1, 7, 12, 14–16] We have been able to confirm these reports in the effect of sodium fluoride on the changes in dog blood.

However, although sodium fluoride, in the proportion of 8 mg. per 100 cc. effectively prevents glycolysis in goat blood, it is, in this case, apparently without effect upon the behavior of the inorganic phosphate. As may be seen by reference to Fig. 3, the curves

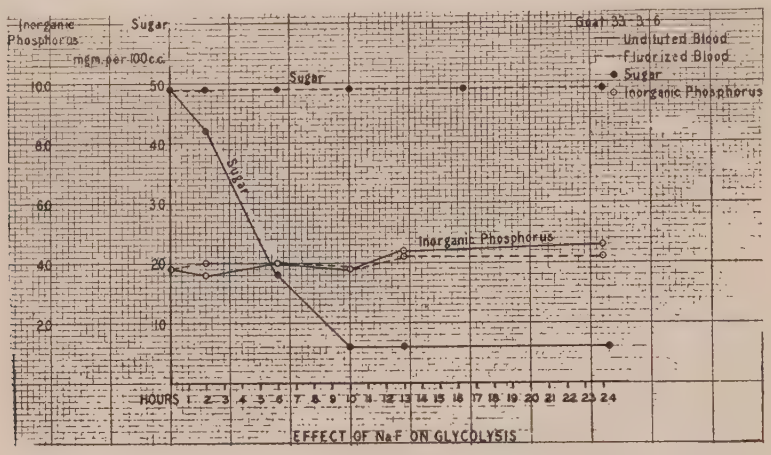

FIG. 3.

Effect of sodium fluoride on glycolysis and changes in inorganic phosphate in goat blood.

illustrating the changes in the level of the inorganic phosphate are entirely similar in the portion of the sample where glycolysis is taking place normally, and in that where it has been suppressed by sodium fluoride. There is, in both cases, only the usual slight gradual rise. Any slight differences in the levels in the 2 portions of the samples are well within the limits of experimental error.

Further data are necessary before these results can be explained satisfactorily. The author is, however, unable at present to pursue the subject further. The results are therefore offered without the additional data necessary for their explanation.

Summary. The changes in the level of the inorganic phosphate which accompany glycolysis in the blood of the dog, the rabbit and the human do not occur in the glycolysing blood of the goat. The addition of glucose to the glycolysing blood of the goat produces only a slight retardation of the gradual rise which normally occurs. The addition of sodium fluoride to the blood of the goat is apparently without effect upon the behavior of the inorganic phosphate.

[1] Barrenschien, H. K., and Braun, K., *Biochem. Z.*, 1931, **231**, 144.

7912 C

Factors to be Considered in Immature Female Rat Titration of Pregnancy Urine.*

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The induction of precocious sexual maturity in the female rat or mouse has been used extensively as the foundation for both qualitative and quantitative studies of the gonadotropic activity of blood and urine and of extracts made from these and other substances. Considerable difficulty has been experienced in this and other laboratories in the application of reactions associated with this maturity phenomenon. For the purpose of evaluating several of the criteria suggested for use in estimating the gonad-stimulating potency of urine, an extended series of titrations of pregnancy urines was conducted in immature female rats. Analysis of the data yields information concerning the relative sensitivity of ovarian, vaginal, and uterine reactions; the conditions limiting their reliability and indications for their use, separately or in combination, as endpoints.

Two lots of urine were titrated. One of these (P.U.A.) was a composite of specimens from pregnancies of 2 to 12 weeks' duration. The other (P.U.B.) was a complete 24-hour collection obtained at approximately the seventh week of gestation in a woman with no evidence of malignancy or endocrinopathy. The follicular hormone was extracted with ether. Doses of less than 1 cc. of urine were prepared by so diluting the stock that each lower dose contained one-half as much urine as the preceding one. The test doses were made up to a volume of 5 cc. per animal. Female rats, of a highly inbred strain, weighing 35 to 45 gm. at 24 days of age were given daily subcutaneous injections for 5 days. During the injection period, observations were made for vaginal opening with estrus. The degree and character of uterine and of ovarian response was recorded at necropsy on the sixth day.

It may be seen (Table I) that, although the higher injection levels caused marked ovarian weight increase in all members of the test groups, the percentage of positive vaginal responses was less than that elicited by smaller amounts of urine which induced far less ovarian weight increases. With reduction of urine concentration,

*Aided by a grant from the National Research Council Committee for Research in Problems of Sex, administered by E. L. Sevringhaus.

TABLE I.

Test Urine	Injection Level (cc.)	No. Rats Used	Vaginal Response No. Open	Vaginal Response No. Not Open	Ovarian Response (wt. in mg.) Range	Ovarian Response (wt. in mg.) Group Average
None (Controls)		12	0	12	10.0-17.8	13.32
P.U. A	5.00	12	6	6	34.0-68.0	49.47
"	3.00	12	9	3	26.0-72.2	42.48
"	2.00	12	11	1	28.1-44.0	37.10
"	1.00	12	10	2	24.0-52.0	37.93
"	0.50	12	12	0	16.0-30.0	22.15
"	0.25	12	12	0	11.0-22.7	17.50
"	0.125	12	12	0	20.1-29.2	24.05
"	0.0625	12	11	1	10.0-16.6	14.08
"	0.0312	12	2	10	9.0-17.0	13.20
"	0.0156	12	0	12	9.0-13.3	11.50
P.U. B	5.00	6	3	3	28.3-61.5	39.60
"	1.00	6	2	4	56.0-64.8	60.05
"	0.50	6	6	0	32.8-34.0	40.50
"	0.25	6	6	0	19.5-35.5	26.00
"	0.125	6	6	0	11.1-27.0	21.36
"	0.0625	6	5	1	13.8-23.3	17.63
"	0.0312	6	6	0	15.9-25.5	21.40
"	0.0156	6	6	0	15.0-26.6	20.01
"	0.0078	6	0	6	13.5-18.1	15.55
"	0.0039	6	0	6	14.0-22.0	16.15
"	0.0019	6	0	6	15.7-20.0	17.13
"	0.00038	6	0	6	11.0-13.6	11.78

P.U. A: Pooled specimens from cases of pregnancy of 2 to 12 weeks' duration.
P.U. B: 24-hour specimen from one case of normal pregnancy of approximately 7 weeks' duration.

the increase in group percentage of positive vaginal responses rather closely parallels the increase in follicular elements and the decrease in hypertrophic lutein bodies in the ovaries.

If the ovaries of the P.U.A. series are arranged in order of weights, there are 11 pairs with weights of 50 mg. or more. In one instance only was estrus associated with ovaries of this weight. In 65 rats whose ovaries weighed 20 to 49 mg., positive vaginal reactions were elicited in 64 animals (98%). Below 25 mg. ovarian weight, estrus occurred in all animals until well within the normal weight range. Examination of the ovaries showed that excessive weight and massive luteinization are to be correlated with failure of estrus. When the vaginal reaction occurred in animals with heavy ovaries, there were always visible follicles. It is notable that a significant number of rats gave the estrus response without increased ovarian weight, that is, evidence of functional activity in normal sized ovaries.

The results of the titration of the 24-hour urine P.U.B. differ but little from those obtained with the P.U.A. batch of pooled urines. There is a more abrupt appearance and disappearance of the vaginal

reaction in rats receiving P.U.B. This is probably due to the smaller number of animals in the test groups. The most outstanding difference seems to be related to the potency of the 2 lots. The highest doses of P.U.B. actually caused less weight increase of ovaries as well as fewer estrus responses.

It is to be noted that, at the lowest injection levels of these test urines, neither ovarian nor vaginal changes could be detected although the uteri of several of the rats were extremely hypertrophic with transparent, greatly distended horns which were filled with fluid.

TABLE II.

		End Points			
		Vaginal Opening and Estrus		100% Ovarian Weight Increase (20-35 mg. ovaries)	
Urine Tested	Injection Level (cc.)	Positive (% of Test Group)	R.U./Liter Test Urine	% of Test Group	R.U./Liter Test Urine
P.U. A	0.50	100		66⅔	2,000
"	0.25	100		16⅔	
"	0.125	100		100	8,000
"	0.0625	92	16,000	0	
"	0.0312	16		0	
P.U. B	0.125	100		83⅓	8,000
"	0.0625	83⅓		33⅓	
"	0.0312	100		66⅔	
"	0.0156	100	64,000	66⅔	64,000
"	0.0078	0		0	

The data assembled in Table II permit consideration of the vaginal reaction and of the ovarian weight response as end-points for the estimation of the gonadotropic potency of P.U.A. and of P.U.B. in rat units per liter (R.U.L.). Katzman and Doisy,[1] in defining their vaginal opening-estrus criterion of activity, include neither the number of rats used per test group nor the percentage response required. The concentration of active substance in P.U.A., therefore, cannot be stated with certainty in Katzman-Doisy units, since with the use of 12 rats per test group, failure to induce vaginal response is more gradual than in the case of the 6 animal titration groups used in the P.U.B. series. If the Hamburger[2] requirement that the unit dose elicit estrus in at least one-half of the injected animals be applied, the potency of P.U.A. may be estimated at 16,000 R.U.L. and that of P.U.B. at 64,000 R.U.L.

The data concerning the ovarian weight end-point (Table II) il-

1 Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 751.

2 Hamburger, C., *Acta Path. et Microbiol. Scand.*, Supplement XVII, 1933.

lustrates the difficulty of using this response alone for determining the unit concentration of gonad-stimulating substance in pregnancy urine. In the titration of P.U.A., the injection of 0.25 cc. induced 100% ovary weight increase in but 16 2/3% of the rats, while twice as much of the urine effected this degree of ovarian growth in 2/3 of the test group. Such results might justify the acceptance of the latter dose as the minimal effective dose, had the titrations not been continued at higher dilutions. The 100% ovary weight increase again appeared in all of the rats receiving 0.0625 cc. of P.U.A. but was not encountered at 4 lower injection levels. Similar results were obtained in the P.U.B. titration series. Comparison of the ovarian morphology of the rats injected at the 2 levels of positive ovarian weight response in each series indicates that 0.5 cc. of P.U.A. and 0.125 cc. of P.U.B. are the lowest levels at which discrete corpora lutea are seen. The highest dilutions of these urines (0.0625 cc. P.U.A. and 0.0156 cc. P.U.B.) to produce 100% ovary weight increases are also correlated with the final detection of follicles of macroscopic size. Thus the luteinizing activity of P.U.A. would appear to be 2000 R.U.L. and that of P.U.B. 8000 R.U.L., while their respective follicle-stimulating capacities would be 8000 R.U.L. and 64,000 R.U.L.

In the titration of P.U.A., the vaginal response serves as a somewhat more sensitive indicator of functioning follicles in the ovaries than does the increased weight with development of follicles of macroscopic size. In the P.U.B. series, these 2 end-points are in perfect agreement.

Summarization of the findings of these titration studies of the gonadotropic effects of graded doses of 2 batches of pregnancy urine leads to several conclusions. (1) Failure to induce vaginal opening with estrus may be due to the injection of too little active material or to inhibition of follicular development by rapid and excessive luteinization. The cause of negative vaginal reactions, therefore, should be investigated by ovary observation. (2) Vaginal opening with estrus is an indication of follicular activity. No information as to the relative follicle-stimulating or luteinizng potency of urines can be gained by means of the vaginal reaction alone. (3) The 100% ovarian weight increase fails at the levels just below the minimum dose required to produce discrete corpora lutea but reappears at a lower urine concentration, which also represents the minimum dose for visible follicles. Failure to induce 100% ovary weight increase may indicate urine concentration inadequate for luteinization or injection of amounts insufficient to produce enough follicu-

lation to effect the ovary weight. (4) The follicle-stimulation dose, as determined by ovary weight and development of follicles to macroscopic proportions, approximates the unit for follicle stimulation as determined by vaginal opening with estrus. Hence, the latter end-point may be used alone for testing dilutions known to be below the level of the minimum luteinizing dose. Such a combination of these 2 end-points may be used for the estimation of the relative concentration of the luteinizing and follicle-stimulating potentialities of test urines. (5) The uterine reactions to given doses of pregnancy urine are extremely variable so cannot be incorporated into the criteria of the unit dose. However, uterine hypertrophy in animals giving neither vaginal nor macroscopic ovarian responses should be considered as indication for microscopic investigation of the ovaries.

7913 P

Theelin and Progestin Injections on Uterus and Mammary Glands of Ovariectomized and Hypophysectomized Rabbits.

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The work of Gardner and Turner[1] suggests that the immediate stimulus for mammary growth is due to the ovarian hormones, theelin and progestin. But Corner[2] and others have obtained growth with anterior pituitary preparations in rabbits which have not been exposed to the influence of corpora lutea. The possibility arises that the ovarian hormones act through the pituitary. To test this point we ovariectomized 40 rabbits and kept them for 2 months to exhaust their supply of ovarian hormones. Then by the use of Firor's[3] technique we attempted to remove their pituitaries, We were completely successful in 10 cases; of these successes, 2 occurred while we were perfecting our techniques, 4 died during the 2 months after the operation before we commenced injections. This left us with 4 successful operations which were used for injection work. We sectioned the contents of the sella turcica in each case after the

1 Gardner, W. U., and Turner, C. W., *Mo. Ag. Exp. Sta. Res. Bull.* 196, 1933.
2 Corner, G. W., *Am. J. Physiol.*, 1930, **95**, 43.
3 Firor, W. M., *Am. J. Physiol.*, 1933, **104**, 204.

experiments. Doctor B. F. Kingsbury kindly examined the sections and confirmed the fact that the pituitaries had been completely removed.

Two months after the second operation all the rabbits were injected daily with 25 R.U. of Progynon B (kindly supplied by Doctor E. Schwenk of the Schering Corporation) and of 0.5 cc. of Progestin (equal to about 4 rabbit units) made in this laboratory. These injections were made over a period of 15 days and the rabbits were then killed. The dosage used was found by Turner and Frank[4] to be effective in producing mammary development in the ovariectomized rabbit, but it cannot be considered ideal for causing progestational proliferation of the uterus. When the rabbits were killed, the mammary glands were dissected out on their muscular plates, trimmed of fat and weighed. Later, they were stained with hematoxylin, stripped from the muscle and mounted. A single ovariectomized rabbit was kept as an uninjected control and killed 4 months after the ovariectomy.

The results showed clearly in all cases that mammary development can occur in the absence of the hypophysis as the result of injections of theelin plus progestin. The degree of development was almost the same in these animals as in those in which the hypophysis was not disturbed or was incompletely removed. The weights of the mammary areas in the ovariectomized injected rabbits averaged 34.7 gm. (4 cases) and in the ovariectomized hypophysectomized injected rabbits, 32.2 gm. (4 cases). The difference is possibly due to variations in the area of muscular tissue removed and cannot be regarded as significant, inspection of the glands did not suggest a difference in the degree of development. The weight of a similar area in the ovariectomized rabbit was 28.4 gm.

The uteri of the ovariectomized hypophysectomized injected rabbits showed some progestational proliferation, averaging 2 on Allen's[5] scale. The corresponding figure for the ovariectomized injected rabbits was 3¼. There seemed to be a lessened response in the absence of the hypophysis. But the number of cases is small. It is sufficient at present to state that progestational proliferation can take place to an extent in the absence of the hypophysis. Uterus weights were, for the double operation 5.5 gm., for the single operation 7.3 gm. and for the ovariectomized uninjected control 3.0 gm.

Our conclusions are that the ovarian hormones are capable of

[4] Turner, C. W., and Frank, A. H., *Mo. Ag. Exp. Sta. Res. Bull.* 174, 1932.

[5] Allen, W., *Am. J. Physiol.*, 1930, **92**, 186.

producing some uterine and mammary development in the absence of the hypophysis. This leads us to believe, in the absence of any other likely route, that their influence is direct. This part of the work is clear cut. There may be a greater uterine response with the hypophysis intact, but the numbers are too small to stress this. The results were consistent, however, and one of the hypophysectomized animals which received 3 times the dose of progestin given to the others showed no further development. Yet another ovariectomized rabbit similarly treated (not included in the averages) showed far more uterine development, and possibly a little more mammary development.

Pacific Coast Section

Stanford University Hospital, February 6, 1935.

7914 P

Effect of Raw Pancreas upon Blood Lipids of Completely Depancreatized Dogs Maintained with Insulin.*

I. L. CHAIKOFF AND A. KAPLAN.

From the Division of Physiology, University of California Medical School, Berkeley.

It was shown[1] that in the completely depancreatized dog maintained with insulin the blood lipids were markedly reduced. The most pronounced change occurred in the cholesterol, the esterified portion of which was either completely absent or present to the extent of 2-7% of the total cholesterol. The present report deals with the effects of the ingestion of raw pancreas upon blood lipids. It was found that the addition twice daily of 125 gm. of the gland to the diet of the dogs soon after pancreatectomy not only prevented

TABLE I.
Dog D.O.—Female—Weight 12.0 kg.
Whole Blood Lipids.

Interval since pancreatectomy days	Cholesterol				Total fatty acids	Phospholipid	Total lipid
	Total mg. per 100 cc.	free mg. per 100 cc.	Ester mg. per 100 cc.	Ester % of total	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.
Preoperative	143	113	30	21	324	293	467
75*	205	132	73	36	412	350	617
91†	110	113	0	0	341	281	451
119*	204	135	69	34			
197†	128	114	14	11			

250 gm. of raw pancreas daily were included in the diet immediately after pancreatectomy until the 75th day and from the 91st to the 119th day.

*This blood lipid followed a period in which raw pancreas was included in the diet.

†This blood lipid followed a period in which raw pancreas was withdrawn from the diet.

*The expense of this investigation was defrayed in part by a grant from the Research Board of the University of California, Berkeley. The insulin was generously donated by the Eli Lilly Company.

[1] Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1934, **106**, 267.

the drop but led to a significant rise above normal in all lipid constituents. In these animals the blood lipids could be made to fluctuate by the addition or removal of the glandular tissue in the diet. The constituent affected to the greatest degree by the ingestion of the pancreas was esterified cholesterol, whereas free cholesterol showed the least change. A typical result is shown in Table I. Following the second removal of raw pancreas from the diet, the esterified cholesterol decreased but did not completely disappear from the blood despite the fact that no pancreas was ingested for a period of 78 days.

7915 P

Growth Rate and Variance in the Razor Clam.

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From the School of Biological Sciences, Stanford University.

In discussing the growth of the razor clam *(Siliqua patula,* Dixon) we have pointed out a correlation between the absolute growth rate and the variability as measured by "D", the interdecile range.[1] In this work we used samples of clams representing the normal population of the respective beds. For this reason the numbers in the older age groups were small, and it was not possible to determine the presence or extent of selective mortality which might greatly affect the relation between growth and variance.

Recently we have selected 2 samples of clams, one from Little River, Humboldt County, California, containing 300 specimens, all 7 years of age, and one from Hallo Bay on the Alaskan Peninsula, containing 76 specimens 13 years old. A complete growth record was obtained for each individual by measuring the consecutive annual rings. The mean length and the variance were calculated for each year for the 2 localities, and the tenth, the fiftieth (median), and the ninetieth percentiles were determined for the distribution of sizes at each age. We have, therefore, by this means obtained data which is obviously unaffected by selective mortality.

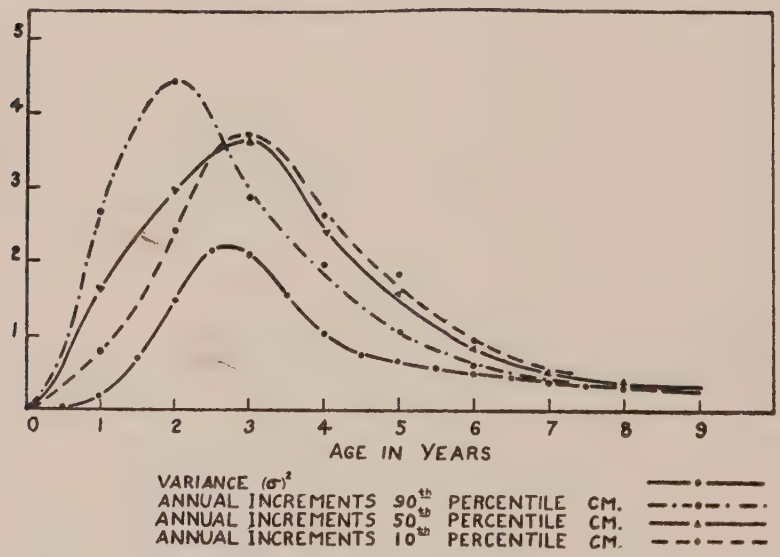
The absolute growth rate (annual increments) shows a high positive correlation with the variance. During the period preceding the inflection of the growth curve both the growth rate and the

[1] Weymouth, F. W., McMillin, H. C., and Rich, Willis H., *J. Exp. Biol.*, 1930, **8**, 228.

variance is increasing, and the point of maximum absolute growth occurs at the same time as that of the maximum variance. As the growth rate declines the variability also declines. As the animals approach final adult size they tend to reach a uniform length.

During the time the growth rate is increasing the successive annual increments are apparently mutually independent. The variability increases with time, in such a manner that we might consider it to be due to chance. If, in the period of declining growth rate, the increments continue to be mutually independent, the variance would rise continually, but at a decreasing rate. A reduction in the variance, such as we find in the present case, may be brought about by a mutual dependence of successive increments.*

If the above relations result from a mutual dependence of the successive annual increments, then those clams showing the greater growth during early life should show lesser growth during the period of declining variance. The accompanying figure shows the increments between successive medians and in the same fashion the



increments for the tenth and ninetieth percentiles. During the preinflectional period the largest clams are growing the fastest. In the postinflectional period the smallest clams are growing most rapidly. In later life the variance remains constant, and clams of all sizes are growing at practically the same rate. If we select groups consti-

*The writers wish to acknowledge the assistance and advice of Dr. Harold Hotelling, of Columbia University, by personal communication.

tuting the largest and smallest clams at the age of 1½ years (approximately those comprised in the ninetieth and tenth percentiles) and follow their subsequent history the results do not differ significantly from those obtained by following the percentiles as above. The smallest group shows the slowest absolute growth up to the point of inflection of the growth curve after which up to 8 or 9 years it grows the slowest.

We have thus clear evidence of compensatory growth in the case of the razor clam, although, of course, the mechanism of regulation is not revealed. It is not possible to attempt an analysis of this phenomenon in a preliminary paper of this character, but we do wish to call attention to the effectiveness of a regulatory process which causes a reduction in the variance to 32% of its maximum value in a space of 2 years.

7916 C

A Comparison of the Resistance of Bacteria and Embryonic Tissue to Germicidal Substances. II. Metaphen.

A. J. SALLE AND A. S. LAZARUS.

From the Department of Bacteriology, University of California, Berkeley.

In a previous communication[1] a comparison was made of the resistance of *Staphylococcus aureus, Eberthella typhi* and embryonic chick heart tissue to Merthiolate and phenol. Toxicity indices were determined by dividing the highest dilution of the germicide that killed the tissue by the highest dilution of the chemical showing no growth of the test organism. Using *Staphylococcus aureus* as the test organism the toxicity index for phenol was found to be 12 and for Merthiolate about 35. It was concluded that phenol possessed a lower toxicity index than Merthiolate when tested by the tissue culture method. Theoretically the smaller the toxicity index the more nearly perfect the chemotherapeutic agent.

In the present paper a comparison was made of the resistance of *Staphylococcus aureus* and embryonic chick heart tissue to Metaphen and phenol. The methods employed were the same as those given in the first paper.

A *Staphylococcus aureus* phenol coefficient was determined for

[1] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 665.

aqueous Metaphen* by the method of Reddish.[2] Phenol killed *Staphylococcus aureus* in a dilution of 1-65 in 10 minutes but not in 5 minutes. The highest dilution of Metaphen killing *Staphylococcus aureus* under the same conditions was found to be 1-6,000. Therefore, the *Staphylococcus aureus* phenol coefficient was 92.

Birkhaug[3] reported a phenol coefficient of 1500. We are at a loss to explain such a great difference in the *Staphylococcus aureus* phenol coefficients of Metaphen when tested by the same method.

Buchsbaum and Bloom[4] reported that Metaphen killed *Staphylococcus aureus* in a concentration of 1-100,000 in cultures of chick periosteal cells. Since their methods differed widely from those employed here no comparisons can be made with their work.

Cultures were prepared from chick heart tissue obtained from 9-day-old embryos. The fragments of tissue were embedded in guinea pig plasma in Carrel flasks. The various dilutions of phenol and Metaphen were made in chick embryonic fluid. The plasma, after coagulation, was washed with Tyrode solution to remove the guinea pig serum, after which the various dilutions of germicide in embryonic fluid were added. Final observations were made at the end of 48 hours. The results are given in Table I.

TABLE I.
Toxicity of Phenol and Metaphen to Chick Heart Tissue and Bacteria.

Germicide	Highest Dilution Showing No Tissue Growth = A	Highest Dilution Showing No Growth of *Staphylococcus aureus* = B	Toxicity Index = A/B	*Staphylococcus aureus* Phenol Coefficient
Phenol	1-840	1-65	12.9	
Metaphen	1-76,000	1-6,000	12.7	92.

The results show that phenol and Metaphen are of the same order of toxicity (12.9 for phenol and 12.7 for Metaphen) when tested by the tissue culture method. Experiments on Merthiolate, as reported previously,[1] showed a toxicity index of 35 by the same procedure and a *Staphylococcus aureus* phenol coefficient of 71.

*The various dilutions of Metaphen used for the determination of the phenol coefficient were prepared by diluting the 1-500 commercial aqueous solution with distilled water.

The various dilutions of Metaphen used for the tissue culture experiments were prepared by diluting the 1-500 commercial aqueous solution with embryonic chick fluid.

[2] Reddish, G. F., *The Newer Knowledge of Bacteriology and Immunology*, E. O. Jordan and I. S. Falk, University of Chicago Press, 1928.

[3] Birkhaug, K. E., *J. Am. Med. Assn.*, 1930, **95**, 917.

[4] Buchsbaum, R., and Bloom, W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1060.

7917 C

Effect of Rice Bran Extract upon Growth of Organisms from Several Genera.

A. J. SALLE AND R. W. DUNN.

From the Department of Bacteriology, University of California, Berkeley.

Salle and Dunn[1] have shown that 25% aqueous ethyl alcohol extracts of rice bran, from which the alcohol was subsequently removed by evaporation, contain a substance which stimulates the growth of *Escherichia coli,* but not *Alcaligenes fecalis.* This substance is related to the carbohydrates, though probably not a hexose, and is possibly identical with the "pantothenic acid" of Williams *et al.*[2]

The action of this extract on *E. coli* led us to suspect that other organisms would also be stimulated. Forty-one organisms, which could be readily cultured in liquid media, were selected. The preparation of the extract, inoculation, and measurement of growth were the same as in the previous experiments.[1] Experiments performed with those to be reported, but using extract prepared by the method of Williams, *i. e.,* extraction of the bran with 60% methyl alcohol, showed both extracts to have identical stimulating properties. The 25% ethyl alcohol extraction was adopted following a suggestion by Dr. S. Lepkovsky that the extraction of large amounts of rice bran might conveniently and fairly inexpensively be made by using the first steps of the method devised by Evans and himself[3] for the extraction of vitamin B.

In all the tables of results, the 3 columns under growth and under final pH show the average values obtained from duplicate determinations, and represent the results obtained with 0.0 cc., 0.1 cc., and 1.0 cc. of extract respectively. The amount of growth, expressed in cubic centimeters, was determined by centrifuging 10.0 cc. of the culture in Hopkins tubes. Original and final pH's were determined by means of the hydrogen electrode. The value for the original pH is the average of the pH of the 2 dilutions of the extract and the pH of the control. The variation of these 3 values was not large enough to warrant tabulation of each in the tables. Inoculation in each case was with 0.2 cc. of an 18-hour culture of

1 Salle, A. J., and Dunn, R. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 168.

2 Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D., *J. Am. Chem. Soc.,* 1933, **55**, 2912.

3 Evans, H. M., and Lepkovsky, S., *J. Nutrition,* 1931, **3**, 353.

the organisms. Aerobic growth determinations were incubated 8 hours, unless designated by an asterisk (*), in which case the incubation time was 24 hours. Gas production during growth is indicated by a dagger (†). The incubation time of the anaerobes was 6 days.

TABLE I.

Organisms	Growth (cc.) 0.0 cc. extract	0.1 cc. extract	1.0 cc. extract	Original pH	Final pH 0.0 cc. extract	0.1 cc. extract	1.0 cc. extract
Escherichia coli	.009	.017	.058†	6.88	6.83	6.70	5.69
Alcaligenes fecalis	.003	.004	.004	7.17	7.32	7.35	7.35
Aerobacter aerogenes	.008	.017	.048†	6.76	6.70	6.55	6.13
Eberthella typhi	.002	.012	.030	6.76	6.78	6.59	5.65
Salmonella paratyphi (para A)	.003	.011	.028†	6.76	6.76	6.60	6.10
Salmonella schottmuelleri (para B)	.010	.018	.044†	6.76	6.79	6.65	5.60
Salmonella enteritidis	.004	.012	.032†	6.76	6.79	6.64	5.89
Salmonella aertrycke	.010	.019	.050†	6.76	6.78	6.62	5.82
Salmonella suipestifer	.005	.015	.030†	6.76	6.80	6.62	6.13
Shigella dysenteriæ (Shiga)	.002	.003	.003	6.76	6.79	6.68	6.72
Shigella paradysenteriæ (Flexner)	.002	.006	.008	6.76	6.80	6.63	6.65
Shigella paradysenteriæ (Hiss)	.003	.009	.015	6.76	6.76	6.59	6.30
*Staphylococcus aureus**	.001	.002	.003	6.85	6.87	6.80	6.80
*Staphylococcus albus**	.003	.006	.029	6.85	6.83	6.71	6.22
*Sarcina lutea**	.002	.002	.002	6.85	6.86	6.85	6.88
*Rhodococcus roseus**	.001	.000	.000	6.85	6.85	——	——
*Gaffkya tetragena**	.002	.006	.005	6.85	6.86	6.73	6.45
*Streptococcus fecalis**	.002	.012	.040	6.97	6.99	6.82	5.60
*Streptococcus lactis**	.001	.005	.060	6.97	6.97	6.85	5.74

Organisms reported in Table I were grown in a medium containing 6.0 cc. of buffer of pH 7.0, 5.0 cc. of infusion medium, extract, and 0.9% NaCl saline to make the total volume 12.0 cc. The aerobic organisms reported in Table II, Group I, and the anaerobic organisms reported in Table III, were grown in a medium containing 11.0 cc. of infusion medium, extract, and saline to make the total volume 12.0 cc. Anaerobic conditions were obtained by placing cultures in a desiccator over alkaline pyrogallol, evacuating, and replacing the evacuated air with hydrogen. A 1% glucose control was run with each of the anaerobic organisms. No observations were made to determine production of gas by the latter. Organisms included in Table II, Group 2 and Group 3, were grown in 5% glycerine-infusion and 5% blood-infusion media, respectively.

Of the 41 organisms reported, the following, according to Bergey, do not ferment carbohydrates: *Alcaligenes fecalis, Brucella melitensis, Brucella abortus, Sarcina lutea, Rhodococcus roseus, Neisseria*

TABLE II.

Organisms	Growth (cc.) 0.0 cc. extract	0.1 cc. extract	1.0 cc. extract	Original pH	Final pH 0.0 cc. extract	0.1 cc. extract	1.0 cc. extract
Group I							
Klebsiella pneumoniæ (Friedlander)	.002	.020†	.040†	7.00	7.00	6.94	5.99
*Bacillus subtilis**	.006	.010	.012†	7.00	7.05	6.98	6.83
Proteus vulgaris	.003	.010†	.040†	7.00	6.86	6.78	6.01
*Brucella melitensis**	.002	.002	.002	7.00	7.00	6.99	7.03
*Brucella abortus**	.002	.002	.002	7.00	6.99	7.00	7.05
*Lactobacillus acidophilus**	.001	.003	.031	7.00	6.95	6.86	6.20
Vibrio comma (cholera)	.010	.017†	.032†	7.00	6.97	6.77	6.25
Vibrio metchnikovii	.009	.017†	.052†	7.00	7.11	6.86	6.47
Group II							
Corynebacterium diphtheria	.007	.009	.020	7.17	6.27	6.28	5.49
Corynebacterium xerosis	.006	.011	.013	7.17	6.72	6.35	6.18
Corynebacterium hofmannii	.001	.002	.005	7.17	5.95	5.64	5.62
Group III							
Streptococcus (type Alpha)	.010	.021	.051	7.38	6.39	5.82	4.41
Streptococcus pyogenes	.012	.018	.040	7.38	6.32	5.77	4.59
Diplococcus pneumoniae	.003	.004	.007	7.38	6.43	5.82	4.92
Neisseria catarrhalis	.023	.021	.023	7.38	7.38	7.64	7.41

catarrhalis, and *Clostridium tetani*. Of this group only *Clostridium tetani* (Table III) was stimulated by the extract. However, glucose also stimulated the growth of this organism, showing that the strain used possesses some carbohydrate-fermentative ability. Reddish and Rettger[4] have shown *Cl. tetani* to have weak saccharolytic properties. *Rhodococcus roseus* (Table I) not only was not stimulated, but failed to grow in the presence of the extract.

TABLE III.

Organisms	Growth (cc.) 0.0 cc. extract	0.1 cc. extract	1.0 cc. extract	1% glucose	Original pH	Final pH 0.0 cc. extract	0.1 cc. extract	1.0 cc. extract	1% glucose
Clostridium welchii	.001	.010	.020	.000	7.00	6.92	7.00	6.70	——
Clostridium histolyticum	.002	.005	.007	.000	7.00	6.96	7.05	7.01	——
Clostridium oedematiens	.004	.013	.025	.013	7.00	7.02	7.01	6.66	6.55
Clostridium sporogenes	.002	.010	.020	.010	7.00	6.88	6.90	6.39	6.37
Clostridium botulinum A	.006	.009	.021	.008	7.00	6.95	6.96	6.52	6.60
Clostridium tetani	.009	.009	.030	.023	7.40	7.39	7.27	6.84	5.57
Vibrion septique	.001	.001	.060	.000	7.00	6.92	6.90	5.76	——

[4] Reddish, G. F., and Rettger, L. F., *J. Bact.*, 1924, **9**, 13.

On the other hand, only one organism which ferments carbohydrates was not stimulated; namely, *Shigella dysenteriae* (Table I). This is probably explained by the fact that the organism is a weak carbohydrate-fermenter, producing acid from glucose only.

In each case, except that of *Clostridium histolyticum* (Table III), stimulation was accompanied by a decrease in final pH, while normal growth showed an increase in final pH.

Clostridium histolyticum, though fermenting several carbohydrates with the production of acid, but no gas, does not ferment glucose, and was therefore not stimulated by it. Since no gas is produced by it from carbohydrates, increased final pH was probably due to excess basic over acidic end-products of metabolism.

For the lack of growth of *Clostridium welchii, Clostridium histolyticum,* and *Vibrion septique* in 1% glucose-infusion medium no explanation is offered. Reinoculation failed to produce growth in any of the 3 cases. Further investigation was not undertaken.

Attempts to purify the extract, which is admittedly a complex material, have been unsuccessful. Due to lack of equipment, the electrolysis method of Williams was not employed.

Conclusion. 1. Since 34 out of 35 known carbohydrate-fermenting organisms were stimulated by rice bran extract, and all 5 of the non-carbohydrate-fermenters were not stimulated, a further confirmation of the conclusion of our first paper on the subject is shown; namely, that the stimulating agent, possibly pantothenic acid, is related to the carbohydrates. 2. A quantitative study of the growth obtained seems to justify the use of rice bran extracts in bacteriological laboratories and related commercial fields.

Missouri Section

St. Louis University School of Medicine, February 13, 1935.

7918 P

Chemical and Optical Properties of Nerve Proteins.

FRANCIS O. SCHMITT AND RICHARD S. BEAR.

From the Department of Zoology, Washington University, St. Louis, Mo.

Previous experiments on thermal shortening and on solvation and desolvation of medullated and non-medullated nerves indicated the presence of oriented protein primary valence chains in these nerves.[1] Similar oriented structures are present in most fibrous tissues.[2] To characterize these structures chemically and to localize them morphologically, experiments have been performed on the chemical and optical properties of normal nerves, of nerves which have been extracted in a variety of ways, and of fibers artificially spun from protein extracts of nerves. The present paper is a preliminary report of this work.

Because of certain difficulties inherent in the procedure of extraction in the case of medullated nerves, and in order to investigate axis cylinder material in as nearly pure form as possible we have turned to non-medullated nerves of the claws and legs of lobsters. The extractions indicate that essentially the same 3 nucleoproteins that McGregor[3] isolated from mammalian central nervous systems are to be found also in lobster nerves. Of particular significance in this connection is the third nucleoprotein extractable at pH 13 to 14. This appears to be the protein which has been called neurostromin by Shkarin.[4] After complete extraction of all proteins soluble below pH 13, the individual finely cut nerve bits still retain their original shape and general structure remarkably. Moreover, such

1 Schmitt and Wade, *Am. J. Physiol.*, 1934, **109**, 93; 1935, **111**, 159, 169.

2 Meyer, K. H., *Biochem. Zeitschr.*, 1929, **208**, 1; 214, 253.

3 McGregor, *J. Biol. Chem.*, 1917, **28**, 403.

4 Shkarin, Inaugural Dissertation, St. Petersburg, 1902, **1**, 453, as quoted by McGregor, p. 421. In many respects neurostromin appears to be similar to the "ellipsin" of Bensley and Hoerr (*Anat. Rec.*, 1934, **60**, 251).

extracted nerve bits display thermal shortening to an extraordinary degree. Although it is impossible to state that other proteins are not involved in thermal shortening of lobster nerve, neurostromin must play the major rôle in the phenomenon.

While it has long been known that fresh lobster nerves show fairly strong positive birefringence we have found that after complete extraction in M/100 NaOH, the remaining neurostromin residue is equally if not more strongly positively birefringent. Extraction with stronger alkali attacks the neurostromin and brings about complete solution of the residue. Coincident with this destruction of the protein is the disappearance of birefringence, although thermal shortening persists as long as there is any visible structure.

Göthlin[5] observed that when lobster claw nerves are immersed in glycerin the birefringence rapidly changes from positive to strongly negative. We have confirmed and extended these observations and find that the reversal may be obtained equally strikingly with neurostromin. Soaking in alcohol prevents this reversal with neurostromin as well as with normal nerve.

It is well known that long asymmetric protein micelles or macromolecules tend to aggregate into fibers when spun from fine capillary tubes into precipitating media. Fibers spun from myosin for example, have been shown to have the same birefringence and to give the same X-ray diffraction pattern as normal muscle.[6] In the case of nerve proteins we have obtained fibers from both of the alkali-soluble nucleoproteins by forcing the alkaline solutions through fine capillaries into dilute acetic acid. Fibers thus made are weak and difficult to manipulate. However, by spinning the proteins into an acetic acid solution containing 30-50% alcohol, well formed fibers have been obtained. Denaturation does not occur under these conditions since the fibers remain soluble in dilute alkali and the proteins are precipitable again by acid. While the freshly formed wet fibers are only weakly birefringent, they show strong positive birefringency when dried. The ease with which these protein extracts may be spun into fibers substantiates the above thermal and optical evidence of the presence of long asymmetric micelles or macromolecules in the axis cylinder of normal nerve. It is not surprising that the thermal shortening, swelling, etc., of spun fibers is not comparable to that of normal nerve since the alkali treatment required for the extraction of the proteins probably has a destructive effect upon certain of the side chain linkages

[5] Göthlin, Kungliga, *Svenska Vetenskapsakademiens Handingar*, 1913, **51**, 1.
[6] Weber, *Ergeb. d. Physiol.*, 1934, **36**, 109.

which bind the primary valence chains laterally. How successfully methods can be devised for spinning fibers which shall have the same thermal and optical properties as normal nerve remains to be determined.

7919 C

Experimental Hypersensitiveness to Staphylococcus.*

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From the Oscar Johnson Institute and Children's Hospital, Washington University, School of Medicine, St. Louis.

The occurrence of type-specific skin reactions to carbohydrates in patients with Staphylococcus infections[1] presented the opportunity for studying experimentally hypersensitiveness to Staphylococcus and its constituents. The recent demonstration that this organism is separable into two distinct immunological types,[2] A and B, dependent upon the presence of chemically and serologically different polysaccharides, has made it possible to consider the influence of type- and species-specificity on the reactions of increased tissue sensitivity. Both monkeys and rabbits were studied to observe evidence of hypersensitiveness to Staphylococcus and its derivatives.

Monkeys *(M. rhesus)* were given injections of Type A or Type B organisms. In one experiment 4 monkeys were given 9 injections intracutaneously of heat-killed bacteria, repeated at weekly intervals. The injections caused only small nodules at the site of inoculation, and the successive reactions following the repeated inoculations were of approximately the same size and severity. In a second experiment, 4 monkeys were inoculated with live bacteria into a subcutaneous agar focus. This was repeated at 3 different times after the effects of the succeeding inoculation had healed completely. The animals in both experiments were skin tested to Type A and B carbohydrates at different periods during the course of observation, but at no time was skin reactivity elicited despite the employment of graded dilutions of carbohydrate. At the termination of the experiment skin tests to carbohydrates were again repeated using 0.2 cc.

*Conducted under a grant from the Commonwealth Fund of New York, N. Y.

1 Unpublished data.

2 Julianelle, L. A., and Wieghard, C. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 947.

quantities of dilutions of 1:10,000, 1:25,000 and 1:50,000. In one animal, injected preliminarily with live Type A Staphylococcus by subcutaneous agar focus, skin reactions were observed to dilutions of specific carbohydrate of 1:10,000 and 1:25,000 but not to 1:50,000. The reactions appeared within 5 to 10 minutes and they were of the wheal and erythema variety. They reached a maximum in about 30 minutes and regressed completely in less than an hour. A comparable reaction to specific carbohydrate has been observed in patients during convalescence following Staphylococcus infections. In the other animals the tests evoked no response. In addition to the carbohydrate tests, injections were made with Staph. protein (0.2 cc. of a 1:10,000 dilution) and 0.2 cc. of Staphylococcus toxin prepared according to the method described by Burky.[3] These injections caused no reactions either immediate or delayed. Blood serum taken from the animals preceding the skin tests were subsequently tested for precipitins for both the protein and carbohydrate. The sera were completely devoid of precipitating antibodies. Blood was also taken at different intervals during the experiments to determine any changes in total protein, globulin, albumin and non-protein nitrogen. The results of the analyses indicated that these constituents remained within the limits of normal variation.

Rabbits were studied in a manner similar to that described for the monkeys. Injections of heat-killed Staphylococci were made intracutaneously at weekly intervals for 6 weeks. One group of animals received Type A while another received Type B. As previously brought out in the case of similar experiments with pneumococci,[4] the reactions at the site of injection were generally larger and of greater intensity with succeeding injections of a constant quantity of organisms, until the 4th or 5th injection. While more marked with Type A organisms, the same phenomenon was observed with the Type B strain. In one of 6 rabbits, a secondary reaction was observed to Type A, following complete disappearance of the primary reaction to the first injection.

In a second experiment conducted concurrently with the former, other rabbits were injected with live Staphylococci into a subcutaneous agar focus. The reaction to Type A was extremely violent, while that to Type B was relatively mild. This is in accordance with the observation that Type A strains are virulent, while Type B strains are not. A second injection was made in the same manner

[3] Burky, E. L., *J. Immunol.*, 1933, **24**, 93.

[4] Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 463.

about a month later when the effects of the primary injection were no longer visible. The response to the second injection of live bacteria was much less marked in the case of both types indicating the acquisition of a definite immunity following the first inoculation.

On one occasion during the course of injections the rabbits were skin tested for sensitivity to both carbohydrates but no reactions were observed. Finally, in terminating the experiment, the animals were tested again to carbohydrate in dilutions of 1:10-25-50-100-200 and 500 thousand. There was no reaction either immediate or delayed. The animals were then tested as described in the case of monkeys to Staph. protein and toxin respectively. All the rabbits reacted in varying intensities to these 2 antigens with a delayed inflammatory reaction. The rabbits were further tested for sensitivity by the more delicate method of direct intracorneal injection of carbohydrate. On the same day one eye was tested with a dilution of 1:25,000, and the other eye with 1:50,000. Since no reaction occurred within 48 hours, the eyes were retested to dilutions of 1:10 and 1:100 thousand, and 4 days later to 1:200 and 1:500 thousand. Two rabbits, one Type A, another Type B, gave clouding and vascularization (pannus) to 1:100,000 dilution. From the nature of the reaction, however, it was considered as a toxic, rather than a hypersensitive response. Final bleedings were made prior to skin testing. It was found that none of the sera contained precipitins for the specific carbohydrate, although all the animals showed varying titers of protein precipitins.

Thus, it is seen that under the conditions specified monkeys are sensitized with great difficulty to Staphylococcus and its derivatives. There occurred no increased reactivity of the skin to the whole organism, since the reaction at the site of inoculation of the bacteria remained approximately constant following each injection. In only one animal was sensitivity to type-specific carbohydrate manifested, and in no instance was a similar state observable to the protein or toxin of Staphylococcus. Despite prolonged inoculations, the sera of the monkeys failed to show precipitating antibodies for either carbohydrate or protein.

Under similar experimental conditions rabbits also proved resistant to sensitization to the carbohydrates of Staphylococcus. All the animals acquired an increased reactivity to the whole organisms, but none of the animals studied reacted to any of the dilutions of polysaccharide used for skin testing. On the other hand, all the animals became hypersensitive to the protein and toxin. Similarly, the sera of these animals while lacking precipitins for the carbo-

hydrates, showed varying titers of protein antibody. As has been shown with Pneumococcus[7] and Streptococcus,[8] this is another example of the difficulty of stimulating type-specific antibodies by intracutaneous injections.

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5 Andrewes, C. H., Derick, C. L., and Swift, H. F., *J. Exp. Med.*, 1926, **44**, 35.

6 Julianelle, L. A., Morris, M. C., and Harrison, R. W., *J. Immunol.*, 1934, **26**, 267.

7 Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 441.

8 Seegal, D., Heidelberger, M., and Jost, E. L., *J. Immunol.*, 1934, **27**, 211.

Southern Section

Tulane University, February 15, 1935.

7920 C

Studies on Animal Transmission of Lymphogranuloma Inguinale.

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Recent researches have shown that Lymphogranuloma Inguinale as described in the continental countries, and Climatic Bubo, as observed in the tropical and subtropical regions, are identical diseases caused by a filtrable virus which can be transmitted to various animals (Findlay[1]). Although this disease has "so often been described in the United States in the past few years that it can no longer be considered as rare" (Stannus[2]), only little experimental demonstration of the causative virus has been yet brought forward (Grace and Suskind[3]). In our present report the possibility of animal transmission of local virus strains was studied. Seven cases of Climatic Bubo observed and diagnosed in the clinics of the Charity Hospital of New Orleans were used for this purpose. All the patients were colored laborers who had spent all their lives in New Orleans or its immediate vicinity, so that the possibility of an infection with a foreign virus, as it is so commonly observed among sailors, was very remote. The diagnosis of Climatic Bubo or Lymphogranuloma Inguinale was made clinically from the appearance of the inguinal buboes and was confirmed in every case by the positive intracutaneous reaction of Frei and the typical microscopic picture of the excised gland.

For the purpose of the transmission of the virus a 20 per cent emulsion of the excised lymph gland was prepared with sterile physiological saline solution and injected into various animals, except in one series of experiments in which the pus of the bubo

1 Findlay, G. M., *Trans. Royal Soc. Trop. Med. and Hyg.*, 1933, **27**, 35.

2 Stannus, H. S., *Trop. Dis. Bull.*, 1934, **31**, 437.

3 Grace, A. W., and Suskind, F. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 71.

was used. The sterility of the emulsions was tested by smear and culture and in some cases the emulsion was passed through a Berkefeld filter. White mice, monkeys (*Macacus rhesus* and *Hapale penicillata*), guinea pigs, chickens and frogs were used in our experiments. The gland emulsion or the diluted pus was injected intracerebrally, intraperitoneally and subcutaneously into the inguinal region and into the prepuce. The intracerebral dosage in mice, chickens, and frogs varied between 0.01 and 0.03 cc., and in monkeys was 0.2 cc. For the subcutaneous and intraperitoneal injections correspondingly larger amounts of virus emulsion were used. The inoculated animals were kept under observation and killed at intervals of one week to one month, to secure virus for further passage.

For the passage from animal to animal a 20% brain emulsion of the infected animal proved most satisfactory. Virus transmission with the emulsion of other organs succeeded with the spleen and heart blood of the infected animals, but failed with the liver and kidney. A brief summary of our results is listed in Table I.

TABLE I.

Material	Diagnosis — Frei Reaction	Diagnosis — Biopsy	Transmitted to	Results
L20 (Bubo)	Positive	Positive	Mice, monkeys, guinea pigs	Pos. 11 passages
L21 "	"	"	Mice	" 2 "
				Neg. 3 "
L24 "	"	"	"	Pos. 3 "
L26 "	"	"	"	" 7 "
L26 (Pus)	"		Mice, monkeys	Negative
L27 (Bubo)	"	"	"	Pos. 7 "
L31 "	"	"	"	" 2 "
L32 "	"	"	"	" 2 "

In each of the 7 cases of Climatic Bubo, the virus could be transmitted into animals and, up to the time of this report, as many as 11 successful passages could be effected. The virus of case L21 lost its virulence after the third successful passage. Although the gland emulsion of L26 proved infective for mice and could be kept through 7 passages, inoculation with the pus which had been obtained by sterile puncture of the gland gave negative results.

The most reliable animal for the transmission of the virus proved to be the white mouse. Symptoms of disease begin to manifest themselves in the inoculated mice towards the end of the first week. They lose their appetite and the fur takes on a ruffled appearance. Later paralysis of the extremities, and occasionally convulsions appear, and in a large number of cases, spontaneous death occurs. The majority of the mice in our experiments, however, were killed as soon as the first cerebrospinal symptoms became manifest, since

one to 2 weeks seemed to us the optimum interval for continued passage of the virus. The meninges of the successfully inoculated animals showed at autopsy a marked injection and the brain appeared soft and edematous. In the positive cases microscopic study of the brain showed as early as one week after inoculation typical round cell infiltration of the meninges and of the perivascular spaces of the brain. A focal increase of large glial cells in the cortical zone of the brain in the vicinity of the inflammatory lesion could be observed in many instances.

The *Macacus rhesus* proved to be the less useful of the 2 species of monkeys used in our experiments. The common Marmoset (*Hapale penicillata*) developed within 1-2 weeks extensive paralysis of the extremities with convulsions, and the autopsy showed the same type of meningo-encephalitis as in the mouse brain. It was interesting to note, however, that as the rule the histological changes in the brain and in the meninges of the monkeys were less severe than in the mice, although the latter showed less marked cerebral symptoms than the monkeys.

Half the guinea pigs inoculated in the groin developed within 5 to 7 days a palpable enlargement of the inguinal lymph nodes and at autopsy showed purulent material in the gray and swollen glands. While the histological picture in many aspects resembled tuberculosis, occasional small abscesses with polymorphonuclear leucocytes were found as well as a hyperplasia of the endothelial cells.

Chickens and frogs inoculated with similar material did not show any symptoms, nor were any gross or microscopical lesions found in the brain at one week and 2 week intervals.

Successful transmission of the same strain of the virus to animals of a different species was possible at will, provided that the species was susceptible to the disease. In our laboratory the virus of L20 could be transmitted from mouse to monkey, from monkey to mouse, back again to monkey and finally from monkey to mouse without losing its virulence.

Conclusions.—The causative virus of 7 cases of Climatic Bubo has been successfully transmitted to mice, monkeys and guinea pigs. Inoculation of chickens and frogs was not successful. The white mouse and the common marmoset proved to be the most useful experimental animals for transmission of the disease. Transmission of the virus from one susceptible species to another was possible and could be repeated at will without apparent change in its virulence. From these experiments it seems reasonable to conclude that the virus of the disease commonly found in the colored population of New Orleans is identical with the virus of the disease described in tropical and continental countries.

7921 C

Usefulness of Organ Emulsions of Infected Animals in Diagnosis of Lymphogranuloma Inguinale.

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To make available a larger supply of L. I.* antigen for clinical and laboratory investigations than could be obtained by the method of Frei,[1] and completely to eliminate the possibility of an occasional false positive intradermal reaction with the use of human material for antigen, we have produced experimental meningo-encephalitis in white mice and monkeys with the virus of lymphogranuloma inguinale, after the method of Hellerström[2] and of Levaditi[3] and his coworkers. The diluted sterilized brain emulsions of both white mice and monkeys (marmosets) have consistently given intradermal reactions, comparable to those obtained with antigens prepared from acute L.I. inguinal buboes after the method of Frei.

The original source of the virus is pus from an acute inguinal bubo in a patient with a positive Frei reaction. The excised lymph nodes must present the characteristic histologic changes of L.I. The lymph gland emulsion may be used in the event that little or no free pus is available. With the finely divided sterile suspension of this material in physiological saline, white mice and monkeys are inoculated intracerebrally. For mice, .01-.02 cc. are used, and for monkeys, 0.2-0.3 cc. The animals employed are the white mouse and the common marmoset (*Hapale penicillata*), which are highly susceptible to infection with the virus of lymphogranuloma inguinale. The mice are killed at the end of one week, their brains being pooled for convenience, but the monkeys are permitted to succumb to diffuse meningo-encephalitis (1 to 2 weeks) or are killed when signs of paralysis develop. Microscopic brain sections show a more or less intense meningeal reaction. Bacteriologic cultures must be consistently negative.

For the preparation of antigen, we employ a 20% sterile brain emulsion in physiological saline. The brain emulsion is heated at 60° C. for 2 hours and at the same temperature for one hour the

*L.I. used in text as abbrebiation for lymphogranuloma inguinale.

1 Frei, W., *Klin. Woch.*, 1925, **4**, 2148.

2 Hellerström and Wassén, *Verh. 8th Intern. Kongr. Dermat. u. Syph.*, Kopenhagen, Aug., 1930.

3 Levaditi *et al.*, *Ann. Inst. Pasteur*, 1932, **48**, 27.

following day. For intradermal tests 0.1 cc. of this antigen is employed and the reaction is read after 48 hours as with the Frei test.

The brain of a single marmoset weighing approximately 6.0 gm. will provide 30 cc. of antigen, sufficient for 300 intradermal reactions. Similarly, a mouse brain weighing approximately 0.3 gm. will furnish 1.5 cc. of antigen, sufficient for 15 reactions. We prefer to employ the monkey brain antigen because it is prepared somewhat more conveniently and the cost is not appreciably greater.

Monkey brain antigens prepared in the manner described above have been tested in 350 patients with uniformly satisfactory results. Mouse brain antigens, employed for approximately 150 intradermal tests, have yielded equally satisfactory reactions. The latter checked consistently with those obtained by the method of Frei. In a consecutive series of 450 intradermal reactions obtained with the use of monkey or mouse brain antigens, prepared according to our method, no more than 32 reactions were equivocal, an incidence of 7%. In the negative reactions the response to the animal brain tissue itself was so insignificant that it was often difficult to detect the site of injection. In patients with active or old healed lesions of lymphogranuloma inguinale, the intracutaneous response was an unmistakable tender area of induration and swelling with a red areola varying in size from 1 to 3 cm.

Emulsions of the abdominal viscera of white mice, prepared one week after intracerebral inoculation with L.I. virus, also possess some antigenic potency. Thus, splenic tissue, injected intradermally as antigen in patients with L.I., will give positive reactions, which are not as strong, however, as those obtained with brain emulsion. Similarly, kidney emulsion will give weakly positive or equivocal reactions, while liver emulsion fails to produce any intracutaneous response. For the diagnostic intracutaneous test for L.I. we prefer to use brain emulsions exclusively.

Conclusion.—Brain emulsions of white mice and of monkeys (*Hapale penicillata*) infected with the virus of L.I. provide a large supply of a uniformly potent antigen for the diagnostic intracutaneous test. There is no danger of contamination with pathogenic organisms, particularly of the spore-bearing type, as there may be when pus from spontaneous buboes in humans is employed directly for the preparation of antigen. Finally, the false positive reactions obtained occasionally with the Frei test are completely eliminated by this method.

7922 C

Effect of Continuous Passage of Endamoeba Histolytica through Experimental Dogs.*

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Since the development of a satisfactory technic for the cultivation of *Endamoeba histolytica in vitro*[1] it has been shown[2,3] that such cultivation gradually reduces the virulence of this organism and renders it less and less capable of infecting experimental animals. On the other hand, the effect of its successive passages through the experimental host has remained a matter for controversy. Some workers[4, 5, 6] have claimed that the organism becomes less virulent with passage. On the other hand, Baetjer and Sellards[7] state that in 11 direct transfers of an active inoculum through kittens they increased the virulence of the amebæ. Dale and Dobell,[8] after 43 passages through kittens, concluded that evidence of increased virulence was lacking. Cleveland and Sanders[3] believed that the increased incidence of infection was due to an increased virulence in the bacteria accompanying the amebæ. All serious attempts to solve this problem have been carried out thus far on experimental kittens.

The present study is based on the successful passage of a single human strain of *Endamoeba histolytica* through a direct line of 20 dogs and subsidiary infection of 40 additional dogs. The original inoculum, obtained in February, 1934, from a case of amebic dysentery contracted in Louisiana, contained an abundance of active amebæ which were immediately introduced intracecally into 2 young dogs according to the technic previously described.[9] One of the 2 became infected and from it adequate material was obtained to continue direct passage of the strain to the present time. All subse-

*Aided by a grant from the David Trautman Schwartz Research Fund.

1 Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, **5**, 371.

2 Dobell, C., and Laidlow, P. P., *Parasitol.*, 1926, **18**, 283.

3 Cleveland, L. R., and Sanders, E. P., *Am. J. Hyg.*, 1930, **12**, 569.

4 Werner, H., *Beih. Arch. f. Schiffs-u. Tropen-Hyg.*, 1908, **12**, 419.

5 Hartmann, M., *Arch. f. Protistenkde*, 1912, **24**, 163.

6 Darling, S. T., *Ann. Trop. Med. Parasitol.*, 1913, **7**, 321.

7 Baetjer, W. A., and Sellards, A. W., *Johns Hopkins Hosp. Bull.*, 1914, **25**, 165.

8 Dale, H. H., and Dobell, C., *J. Pharm. and Exp. Therap.*, 1917, **10**, 399.

9 Faust, E. C., *Porto Rico J. Pub. Health and Trop. Med.*, 1930, **6**, 391.

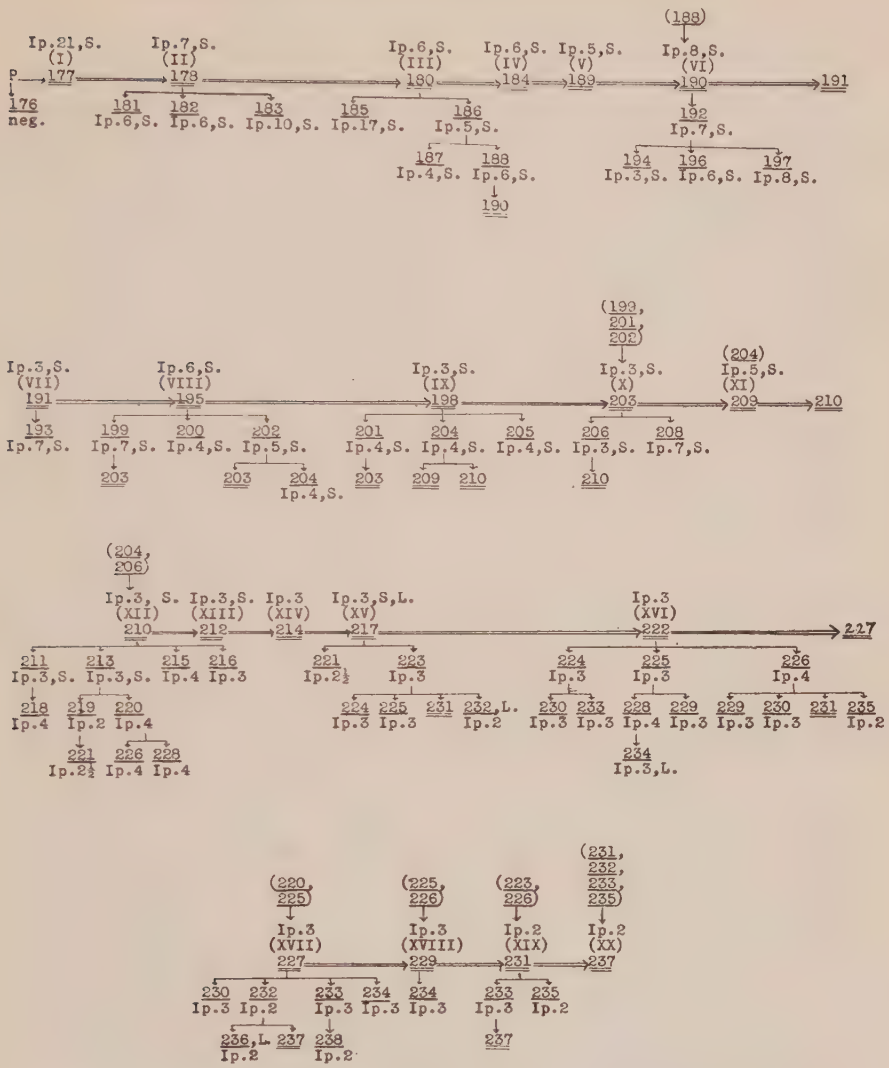

TABLE I.

Showing increased virulence of a human strain of *Endamoeba histolytica* by passage through dogs.

Arabic numerals from 176 to 237 refer to dogs in this series; Roman numerals in parenthesis from (I) to (XX) indicate the particular direct line subpassage from dog to dog.

P, patient from whom the inoculum was originally received.

Ip., incubation period (in days).

S, indicates that the dog was fed tinned salmon before inoculation and during the incubation period.

L, indicates that the dog was fed raw liver following the incubation period.

Series numbers *underlined twice* are in the direct line; those *underlined once* indicate subsidiary lines of subpassage. Arabic numerals in parenthesis above the numbers in the direct series refer to sources of inocula additional to those in the direct line.

quent animals inoculated became infected. All were carefully studied during the course of the infection and all were carefully examined post-mortem. The successive transfers through these animals, utilizing inocula obtained only from living hosts, are diagrammatically represented in Table I. Since all of the inoculated dogs became infected, incidence as a criterion of virulence was *ipso facto* eliminated. Reliance has therefore been placed on other factors, including (1) the length of the incubation period, (2) the degree of pathology induced, (3) the age and size of the animal utilized, and (4) the effect of certain foods as adjuvants in inducing infection.

The incubation or prepatent period is considered terminated[9] as soon as evidence of multiplication of the amebæ and of the earliest host-tissue damage has been obtained by cecoscopic examination. Such evidence may precede clinical amebiasis by several days and in resistant hosts it may be the only evidence of infection ever obtained. But in the present series, except for the first positive animal (No. 177) and one other (No. 185), it never preceded clinical amebiasis more than 48 hours, in 30 % of the cases only 24 hours, and in 60 % was simultaneous with clinical dysentery. During the first 9 months the incubation period was reduced from 7.3 to 3.8 days (average of 10 animals each); later, to 3.25 days, and in the last 10 members of the series, to 2.4 days. Except for the first member of the series (No. 177) all of the first 35 animals were fed tinned salmon during the incubation period.[10] With one exception (No. 217), the later animals had a balanced ration during prepatency. Furthermore, although the early members of the series were all young pups and later in the series larger, more mature and presumably more resistant animals were selected, the incubation period was consistently reduced. On the basis of the incubation period, therefore, the results of amebic infection in our canine series provide consistent evidence supporting the view of Baetjer and Sellards that successive passages of *Endamoeba histolytica* through susceptible hosts enhance the virulence of the inoculum.

The ameba, as originally obtained from the human host, was a medium-sized race. There has never been any tendency toward decrease or increase in the size of the organism. It has apparently propagated entirely by binary fission. Encystation has never been observed in the feces or in the tissues of the infected animals.

Paralleling the reduced incubation period, not only was there an increase in the amount of discharged bloody mucus, but each drop

[10] Faust, E. C., Scott, L. C., and Swartzwelder, J. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 540.

of exudate was richer in numbers of amebæ. Examination of sections of representative lesions has demonstrated a remarkable numerical increase of full-sized individual amebæ in each colony, particularly at the base of the glands. As soon as it was discovered that the incubation period was being appreciably shortened, in order to prevent any possible individual host factor from modifying the results, as well as to insure continuity of the strain, material from 2 or more hosts was frequently pooled and used as inocula. Uncomplicated deaths of the experimental hosts occurred most frequently when the incubation period had been reduced to about 3 days (Nos. 211 to 221, after 9 months' passage). Post-mortem examination of all these dogs except No. 216 showed that practically every available site in the entire length of these dogs' large intestines was beset with typical pinpoint or craterous lesions, and in one animal (No. 214) the posterior ileum was comparably involved. The degree of pathogenicity in these intestines was both quantitatively and qualitatively greater than in the early members of the series. In only 2 of these animals was there any evidence of non-amebic ulceration of the intestinal wall. With further reduction in the incubation period, there was less tendency to deep invasion and more superficial confluent ulceration, still without any generalized inflammation of the wall except in isolated cases (Nos. 230 and 237). Clinically these later members of the series have shown more marked early dehydration and exsanguination.

Cleveland and Sanders[3] and more recently Frye and Meleney[11] believe that the bacteria of the large intestine play some part in the development of amebic lesions. The former investigators stress the importance of the increased virulence of the bacteria passed along with the amebæ from host to host, while the latter workers have concluded from their studies that both the incidence of infection and the degree of pathogenicity for the host are to a certain extent dependent on the associated bacteria. Although our dogs have consistently failed to furnish corroborating evidence, we do not deny the possible secondary rôle which bacteria may play in the development of the amebic process. However, at the suggestion of Col. Chas. F. Craig, to rule out bacteria as the primary agents of amebic colitis in our dogs, we have cultured on beef broth in 250 cc. Erlenmeyer flasks the bacteria taken directly from the intestines of our most fulminating cases of amebiasis (pooled from Nos. 224, 226-229, 231-236) and have repeatedly introduced intracecally large amounts of these inocula into 3 negative pups. Neither dysentery

11 Frye, W. W., and Meleney, H. E., *Am. J. Hyg.*, 1933, **18**, 543.

nor diarrhea has been produced, and at autopsy the wall of the large intestine of each animal was completely free of any inflammatory areas. We conclude, therefore, that the bacteria associated with the amebæ in our inocula have not been responsible for the reduction in the incubation period, for the increased ease with which we have transferred our strain of ameba, or for the increase in the number and degree of severity of the amebic lesions produced.

7923 C

Observations on Antigens for Complement Fixation in Amebiasis.*

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Since the publication of the technique of the complement fixation test for amebiasis devised by the senior author (Craig)[1], in which absolute alcoholic extracts of cultures of *Endamoeba histolytica* were employed as antigens, several modifications of the test have been published and other antigenic extracts have been described.

Menendez[2] and Spector[3] employed simple alcoholic extracts of cultures of *E. histolytica* as antigens with satisfactory results, and Menendez stated that a simple suspension of cultures of *E. histolytica* in formalinized saline also possessed good antigenic properties. Sherwood and Heathman[4] and Heathman[5] used antigens prepared by extracting the dried sediment of cultures of *E. histolytica,* rich in the amebæ, with ether, 96 % alcohol and acetone, after which cholesterin was added in different amounts, and found that such antigens were efficient. Tsuchiya[6] and Weiss and Arnold[7] successfully employed absolute alcohol extracts of cultures of *E. histolytica* in their modifications of this complement fixation test. With all of these antigenic extracts these observers obtained a high percentage of positive results in infections with *E. histolytica* and

*Aided by a grant from the David Trautman Schwartz Research Fund

1 Craig, C. F., *Am. J. Trop. Med.*, 1927, **7**, 225.

2 Menendez, P. E., *Am. J. Hyg.*, 1932, **15**, 785.

3 Spector, B. K., *J. Prev. Med.*, 1932, **6**, 117.

4 Sherwood, N. P., and Heathman, L., *Am. J. Hyg.*, 1932, **16**, 124.

5 Heathman, L., *Am. J. Hyg.*, 1932, **16**, 97.

6 Tsuchiya, H., *J. Lab. and Clin. Med.*, 1934, **19**, 495.

7 Weiss, W., and Arnold, L., *Am. J. Digest. Dis. and Nutrition*, 1934, **1**, 231.

negative results in other infections or diseases and in healthy individuals, thus amply confirming the specificity of the test.

We have recently endeavored to obtain more easily prepared antigens by extracting the mucoid material which may be readily obtained from the intestine of dogs suffering from acute amebic dysentery experimentally produced. Such material is usually very rich in amebæ and contains very few bacteria as compared with cultures of this parasite. The utilization of this material as a source of antigen for the complement fixation test was first suggested by our assistant, Dr. Edwin S. Kagy, and it has been found that extracts prepared from it are suitable for use as antigen and are sometimes stronger in antigenic properties than the absolute alcohol extracts of cultures of *E. histolytica.*

The method of infecting dogs with this ameba has been described by Faust[8] and the exact technique of the complement fixation test by Craig.[9–10] The mucoid material from the intestine of the infected dogs used in the preparation of the antigenic extracts was obtained by aspiration from the cecum and upper portion of the colon, utilizing a glass pipette to which a 25 cc. bulb was attached. The material, so obtained, consisting of bloody mucus, rich in amebæ, has been extracted and fractionated in various ways and the extracts and fractions thereof have been titrated for their antigenic value.

Antigens prepared by extracting the dried residue of the mucoid material with ether and the lipoids precipitated from this extract with acetone, the resulting residue being added to alcoholic extracts of the same material, gave excellent reactions when used in proper amounts but we found that, with one exception, such antigens could not be used diluted. This particular antigen, when diluted with 4 parts of normal saline, gave satisfactory results. Attempts to fractionate this antigen, and other antigens prepared in the same manner, by extracting the ether insoluble portion with water or the insoluble portion of such an extraction with absolute alcohol, resulted in no improvement in antigenic qualities and such fractions were usually either hemolytic or anticomplementary, and of much less practical value than the original antigenic extracts.

More recently simple alcoholic extracts of the mucoid material obtained from the infected intestine of the dog have been investigated and it has been found that the extraction of this material, when it is rich in amebæ, with absolute alcohol, in the proportion of

8 Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 908.

9 Craig, C. F., *Am. J. Trop. Med.*, 1929, **9**, 277.

10 Craig, C. F., *Amebiasis and Amebic Dysentery*, Springfield, Ill., and Baltimore, Md., 1934, page 231.

one part of the material to 7 parts of absolute alcohol, yields an excellent antigen for complement fixation in amebiasis. The extraction is conducted in an incubator at 45° C. for 15 days, the flask containing the material being thoroughly shaken several times a day during that time. After extraction, the mixture is filtered, diluted with from 3 to 5 parts of normal saline and tested for its hemolytic, anticomplementary and antigenic properties.

Antigenic extracts prepared in this simple manner appear to be more active than when the acetone insoluble lipoids are either used alone or added to alcoholic extracts of cultures of *Endamoeba histolytica* or of the mucoid material. It has been found that the dilution mentioned can generally be used with excellent results, whereas in our experience most of the lipoid antigens gave poor results when diluted.

Alcoholic extracts of the mucoid material obtained from the intestine of dogs suffering from amebic dysentery gave fully as good results as extracts made from cultures of *E. histolytica.* Owing to the difficulty experienced by many in the cultivation of this organism, the preparation of antigens from cultures has been abandoned by many laboratories. It has been shown by Faust that dogs are comparatively easily infected with *E. histolytica* and that the infection can be maintained in the laboratory by transmission from animal to animal. Sufficient mucoid material for extraction may easily be obtained from the intestine, in an acutely infected dog, and as this material is usually much richer in the amebæ than are cultures, and much more free from bacteria, it is believed that it furnishes an excellent source of antigen for use in complement fixation in the diagnosis of amebiasis.

7924 C

Effect of Temperature of Storage on Bacteria in Water Samples.

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Bacteriological control of water supplies frequently involves examination of samples which have been shipped considerable distances. All editions of Standard Methods for the Examination of Water and Sewage provide for icing samples during shipment. Berry,[1] Jordan and Irons,[2], Hale and Melia,[3] Albert, Hinman and

Jordan,[4] Eijken,[5] Huss,[6] and Rector and Daube[7] reached conclusions which are not in agreement. Berry found little difference in the *Escherichia coli* count of iced and uniced samples and no appreciable multiplication of this group in stored samples. Boruff and Buswell[8] reported that a sample of water analyzed immediately showed more viable bacteria than after shipment iced about 100 miles (1-2 days in transit). Warm polluted water iced even for 12 hours underwent a distinct decrease in count. Their work would seem to indicate that the type of sample would largely determine the changes which would take place. Icing a sample of warm polluted water might cause more profound changes than icing a sample of cold water in the winter.

The work here reported involved 5 different types of water: (1) polluted surface water; (2) water to which soil had been added; (3) diluted sewage; (4) sterile distilled water inoculated with pure cultures; and (5) tap water inoculated with pure cultures. The technic in general was to divide the sample into 3 portions for storage at 3 different temperatures—in the refrigerator at about 4° C., at room temperature from 20.5 to 21.8° C., and in the 37° C. incubator. These incubation temperatures cover about the range to which samples might be subjected between time of collection and analysis.

1. The total count of polluted surface water increased on storage at room temperature and 37° C. for 24 hours. The total count on the portion stored in the ice box, however, showed little change, only a slight increase being noticed. In this case the advantage of icing is indicated.

2. Samples of water to which soil was added showed little change in the total counts of portions stored under the 3 storage temperatures mentioned above.

3. Counts on sample of diluted sewage increased rapidly on the portion stored at 37° C. Two other portions stored at 37° C., and in the refrigerator showed little change in the number of bacteria.

4. Sterile distilled water was inoculated with *Escherichia coli, Aerobacter aerogenes, Eberthella typhi,* and 2 organisms isolated

[1] Berry, F., *Am. J. Pub. Health,* 1926, **16**, 700.

[2] Jordan, E. O., and Irons, E. E., *Am. Pub. Health Assn. Papers and Reports,* 1899, **25**, 564.

[3] Hale, F. E., and Melia, T. W., *J. Inf. Dis.,* 1910, **7**, 587.

[4] Albert, H., Hinman, J. J., and Jordan, G., *Am. J. Pub. Health,* 1916, **7**, 2, 1010.

[5] Eijken, P. A. A., *Chem. Weekblad,* 1918, **15**, 1519.

[6] Huss, H., *Wasser u. Abwasser,* 1922, **17**, 244.

[7] Rector, F. L., and Daube, H. J., *Absts. Bacteriology,* 1917, **1**, 57.

[8] Boruff, C. S., and Buswell, A. M., *Illinois State Water Survey,* Bulletin 28.

from sewage. All were strains which had not been carried on laboratory media. The strain of *Escherichia coli* died out rapidly when stored in distilled water at the 3 temperatures of storage; death was especially rapid at 37° C. Our results agree quite well with those reported by Hale and Melia. In one case icing seemed to show slight advantage. *Aerobacter aerogenes* died almost completely in 16 hours at all temperatures of storage in one test and within 3 hours in a second. In a third experiment somewhat longer survival periods were indicated. The 2 members of the Escherichia-Aerobacter group isolated from sewage died out quite rapidly except in one portion stored in the refrigerator. *Eberthella typhi* decreased rapidly in distilled water stored at 37° C. and room temperature, while at 3-5° C., significant decrease was not noticed after 48 hours, a period quite beyond that for which a water sample would be stored before analysis.

5. Tap water after sterilization was inoculated with 2 strains of *Eberthella typhi,* 3 strains of *Salmonella paratyphi,* 2 strains of *Salmonella Schottmülleri,* 3 strains of *Shigella dysenteriae,* and 2 strains of *Escherichia coli.* These species possess some significance for sanitary bacteriologists.

Iced samples of tap water inoculated with *Escherichia coli* showed less change in count than did the portions kept at room temperature and 37° C. With one strain of *Eberthella typhi,* however, the counts fell off somewhat in the iced portion but increased in the portion stored at room temperature. The other strain gave inconsistent results indicating that strain differences may affect the results. One strain of *Salmonella paratyphi* died out within 6 hours in iced tap water; another maintained practically the same count under these conditions; the third strain showed distinct decrease under all conditions. Two strains of *Salmonella Schottmülleri* showed little change in number when stored in the refrigerator. Icing was decidedly advantageous, however, with three strains of *Shigella dysenteriae.*

Lactose fermentation was observed after 48 hours in iced samples stored at room temperature and at 37° C. Tap water to which soil had been added, contained lactose fermenting bacteria in dilutions of 1-100,000 after 72 hours in the portions stored at all temperatures; in higher dilutions, however, gas was produced only by iced portions. Other experiments showed that lactose fermenting bacteria disappeared quite rapidly from portions of water stored at the higher temperatures.

The total count (24 hours) of polluted surface water increased

upon storage at room temperature and at 37° C. In the ice box, however, the counts increased but slightly, results on analysis at the end of 24 and 48 hours, comparing favorably with those from immediate analysis. Advantage of icing this particular surface water is thus indicated. Two samples of water to which soil had been added showed slight differences in counts obtained at the 3 temperatures.

In one sample of diluted sewage stored at 37° C., marked increase in count was observed. In a second sample the 37° C. and ice box counts were not widely divergent.

A strain of *Escherichia coli* inoculated into sterile distilled water died out rapidly at the 3 temperatures of storage particularly at 37° C. This is in agreement with results reported by Hale and Melia. In one case icing appeared to be slightly advantageous.

A suspension of *Aerobacter aerogenes* in sterile distilled water died almost completely within 16 hours at all temperatures in one test and within 3 hours in a second. In another the count was not greatly affected in 24 hours, decreased markedly in 96 hours in the portion stored at room temperature, and in 144 hours in the ice box and 37° C. portions. This was the only sterile distilled water sample which did not show sudden and marked decrease in count within 48 hours.

Counts in sterile distilled water samples containing organisms 2 and 5 (isolated from sewage on Endo's agar and shown to be members of the *colon-aerogenes* group) decreased within 3 hours. In 72 hours the count was low (1-2 colonies per cc.) except in the ice box sample containing organism 5.

In sterile water stored at 37° C. and at room temperature *Eberthella typhi* (strain 340) decreased from a high initial count (greater than 300 colonies per plate) to a low count within 20 hours, while at 3°-5° C. a marked decrease was not observed after 48 hours.

Lack of food materials in sterile distilled water may have caused the high death rate of most of the organisms studied in this medium. It probably cannot be attributed to traces of copper because frequent tests indicated absence of this metal. Since tap water contains more food material and is not sterile, it affords conditions more nearly like those normally encountered in natural waters and for this reason was used in the remaining experiments.

Counts in the iced samples of tap water inoculated with *Escherichia coli* compared more favorably with original counts at the end of 24 and 48 hours than did either the sample stored at room temperature or at 37° C.

An iced portion of tap water containing *Eberthella typhi* gave counts at the end of 24 hours which were slightly lower than the original. The room temperature portion had increased in count. At the end of 48 hours; however, the samples incubated at room temperature had increased in count and the ice box sample had decreased.

The counts obtained at the end of 24 hours on tap water inoculated with *Eberthella typhi* packed in cracked ice were much nearer the original than were those obtained from the water stored at room temperature and 37° C. The sample was packed in cracked ice to simulate actual conditions used in practice. This method was discarded, however, since the cracked ice melted rapidly.

It is difficult to reach conclusions from the data obtained with 2 strains of *Eberthella typhi* in tap water. One did not behave consistently. In the first experiment distinct increases in number of cells were obtained after incubation for 144 hours when stored under the three temperatures used in all of the experiments. It is significant to point out, however, that distinct decreases in number of viable cells were obtained after storage for 24 hours. This is perhaps the period of time over which a sample would be held under actual conditions of shipment. The next experiment with this strain gave different results. After 144 hours' storage at the 3 temperatures used, a decrease in viable cells was secured. However, after 24 hours it is probable that the number of viable cells had begun to increase because the 48 count, the first one which was made, was much higher than the initial count. It is difficult to explain the results secured in these 2 experiments. They probably indicate that the actual conditions which happen to exist in the culture during storage have much to do with the behavior of the organisms contained therein. In other words, the conditions which obtain in the sample bottle may be more important than those existing around it.

One strain of *Salmonella paratyphi* died out within 6 hours when stored in tap water on ice; another maintained practically the same count under these conditions, and a third decreased in count. Results with the 3 strains at the end of 24 and 48 hours varied widely. It is probable that these differences in reaction to storage temperatures may be attributed to differences in the strains themselves.

Tap water containing 2 strains of *Salmonella schottmülleri* stored in the ice box increased in count in 24 hours, but the 48 hour counts were about identical with the originals. The 48 hour count on a sample containing a third strain of *Salmonella schottmülleri* compared well with the original. In some cases storage at room tem-

perature and 37° C. resulted in an increase and in others a decrease in number of viable organisms.

According to the results secured with 3 strains of *Shigella dysenteriæ,* icing was advantageous in maintaining the viable cells over the period of the experiments.

Fermentation in lactose broth was observed at the end of 48 hours in polluted surface water stored in the ice box and in the 37° C. incubator but not in samples held at room temperature. In another case gas was observed at the end of 144 hours in only the ice box and room temperature portions. Advantage of icing is again indicated.

Water to which soil had been added contained lactose fermenting organisms in the 1-100,000 dilutions after 72 hours at all 3 temperatures, but in the 1-1,000,000 dilutions gas was produced only at low temperatures.

Gas was observed in lactose broth cultures at the end of 144 hours in water containing *Escherichia coli* at room and ice box temperatures but not at 37° C. In another experiment on the same organism gas was evident in the ice box and 37° C. samples but not at room temperature.

At the end of 96 hours sterile water containing *Escherichia coli* fermented lactose only after storage at low temperatures. The same results were observed at the end of 24 hours with an organism isolated from sewage and at the end of 120 hours with an organism from the same source. The strains of *Escherichia coli* studied died out more readily at 37° C. in most cases.

Conclusions.—1. The characteristics of the particular strains of bacteria present in the water sample have much to do with their behavior during storage. 2. It is difficult to secure comparable counts and perhaps the slight differences up and down which were observed in some of the samples were due to difficulties in sampling. 3. The number of viable organisms present in water after storage for 48 hours in the refrigerator (0°-7° C.) varied but slightly from the original. 4. The ability to ferment lactose is not inhibited by low temperatures of storage but seemed to be maintained over longer periods in iced specimens. 5. The data collected in this investigation indicate in practically all cases that water samples should be kept cold between collection and analysis. This would indicate that icing of samples as provided in Standard Methods for the Examination of Water and Sewage is desirable.

7925 P

Glutathione Concentration of White Leghorn and Barred Plymouth Rock Embryos After Fourteen Days of Incubation.*

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Rhode Island Red embryos, destined to develop into adults of comparatively large size, proliferate cells at a faster rate than White Leghorn embryos, destined to develop into adults of comparatively small size.[1] It is known that glutathione (sulphydryl) stimulates cell proliferation, and that rapidly growing rats and rabbits have a greater concentration of glutathione in their body tissues than less rapidly growing controls.[2-3] Furthermore, fasted new-born rabbits which are destined to develop into large adults have a greater concentration of glutathione than those destined to develop into small adults.[4]

The following experiment was performed in order to determine the glutathione concentration of White Leghorn and Barred Plymouth Rock embryos, and to ascertain whether or not a difference in concentration exists between the two breeds. Most of the Leghorns, and all of the Barred Plymouth Rocks used in the experiment were descendants of the stocks used by Asmundson and Lerner in studies on the inheritance of adult body size. It was definitely known that the Rocks had a significantly greater post-hatching growth rate than the Leghorns.[5]

The range of adult weights of the Leghorn hens used for the production of eggs in the first experiment was from 1418 to 2250 gm., with a mean weight of 1814, and of the Barred Plymouth Rocks, 2481 to 3143 gm., with a mean weight of 2881. There was a considerable variation in the weight of the Leghorn sires, the range extending from 1940 to 2915 gm. with a mean of 2385. The only Rock male used weighed 3669 gm.

In the second experiment the Leghorns used for the production of eggs were more carefully selected for uniformity of adult size.

*This investigation was aided by a grant from the National Research Council for the study of glutathione in relation to growth and hereditary size.

1 Blunn, C. T., and Gregory, P. W., *J. Exp. Zool.* (in press).

2 Gregory, P. W., and Goss, Harold, *J. Exp. Zool.*, 1934, **69**, 13.

3 Goss, Harold, and Gregory, P. W., *J. Exp. Zool.* (in press).

4 Gregory, P. W., and Goss, Harold, *J. Exp. Zool.*, 1933, **66**, 155.

5 Asmundson, V. S., and Lerner, I. Michael, *Poultry Science*, 1934, **13**, 348.

The weight of the females ranged from 1480 to 2130 gm., with a mean of 1703. The Leghorn males varied from 1550 to 1860 gm., with a mean of 1725. The Rock females varied from 2340 to 3220 gm., with a mean of 2698, while the Rock males varied from 3500 to 4150 gm., with a mean of 3841.

The eggs from which the embryos were obtained were selected in pairs on the basis of weight, one Leghorn and one Rock constituted a pair. Most of the eggs were paired within 0.2 gm. However, in a few cases the difference between the weights of the eggs paired was slightly greater. The paired eggs were incubated in the same incubator at the same time, and the glutathione determination of the embryo was made after 14 days of incubation. The general method of analysis has been described.[2, 6] The data were analyzed by Fisher's method of pairing. Table I contains the summarized results.

TABLE I.

Summary of Glutathione Concentration of Barred Plymouth Rock and White Leghorn Embryos Analyzed after 14 Days of Incubation.

Exp. No.		Pairs	Mean GSH mg./100 gm. Rocks	Mean GSH mg./100 gm. Leghorns	Value of t	t value necessary to be highly significant
1	Spring, 1934	40	48.7	46.4	2.594	2.576
2	Fall and winter 1934-35	25	44.7	40.0	3.265	2.787
1 and 2 combined		65	47.2	43.9	4.055	2.576

The Leghorns used in Experiment 1 were rather variable so far as adult size is concerned, but the mean GSH concentration of the Rock embryos was significantly greater than that of the Leghorns. The Leghorns of Experiment 2 had been inbred with selection for weight. As a result the adult size of the females and especially of the males was less than that of the Leghorns used in the first experiment. The Rocks used in the second experiment were descendants of the birds used in Experiment 1. The results of Experiment 2 emphatically confirm the observations of the first experiment, that the embryos of the larger breed have a greater concentration of GSH than those of the smaller. When the data of Experiments 1 and 2 are combined, the results are most significant.

The glutathione values are positively correlated with the rate of cell proliferation at 14 days of incubation. It is also correlated with the post-hatching growth rates and adult weights.

[6] Goss, Harold, and Gregory, P. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 681.

7926 C

Determination of Fermentable Sugar in Muscle.*

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It was noted[1] that short tetanic stimulation of rat muscle resulted in an increase in fermentable sugar, suggesting that some of the split-products of glycogen escaped phosphorylation and remained as fermentable sugar in muscle. Since several intermediates might be formed between glycogen and the first phosphorylized product, it seemed of interest to determine the nature of the fermentable sugar formed as a result of contraction. It was shown previously that heart muscle contained a larger amount of fermentable sugar than resting skeletal muscle. This was attributed to the continuous activity of the former organ and made it desirable to determine the nature of the fermentable sugar in heart muscle as well.

In view of the low concentration of fermentable sugar in muscle, large amounts of tissue had to be used for each experiment. Cats were anesthetized with nembutal and the gastrocnemii dissected free of surrounding tissue for rapid removal. One muscle was stimulated tetanically for four 10-second periods through the sciatic nerve, while the other muscle served for the determination of the resting value. Generally the muscles of 2 cats were combined; these yielded from 40 to 60 gm. of resting muscle and a similar quantity of stimulated muscle.

In some preliminary experiments the muscles were frozen in a CO_2 snow-ether mixture prior to extraction. It was found that the contraction elicited by the freezing resulted in an increase in the fermentable sugar. Hence, this method of fixing the muscles was replaced by one in which the muscles were immersed immediately after removal in N sulfuric acid cooled to about —4°.

It seemed doubtful at first that a muscle of the size of a cat gastrocnemius could be fixed in this manner. In control experiments it was found that the fermentable sugar content of thin cat muscles (weighing 1 to 2 gm.) fixed in cold acid was the same as that of gastrocnemii. In both cases the values were lower than those found in previously frozen muscle. Freezing apparently produces a measurable breakdown of glycogen, because in addition to

*Aided by a grant from the Rockefeller Research Foundation to Washington University for research in science.

[1] Cori, G. T., Closs, J. O., and Cori, C. F., *J. Biol. Chem.*, 1933, **103**, 13.

the increase in fermentable sugar there occurs also an increase in hexosemonophosphate.

After half an hour the weighed muscles were removed from the acid and ground in a meat chopper. The muscle pulp and acid were transferred to a mortar and after grinding with sand the mixture was allowed to extract over night. "Iron and mercury" reagent† was added equal to two-tenths of the final volume and enough water to make the dilution 1:8, assuming the water content of the muscle to be 80 %. The mass was then neutralized with $BaCO_3$, filtered through a Büchner funnel, Fe+Hg removed as sulphides and H_2S and Ba removed. A second precipitation was usually carried out by addition of Fe+Hg reagent equal to two-tenths of the filtrate volume. After treatment as above, the pH of the filtrate was adjusted to between litmus and congo and evaporated to dryness *in vacuo*. The solids were taken up in 2.5 cc. of water and 50 cc. of 95 % alcohol was added to remove salts. After remaining over night in the refrigerator, the salts were filtered off. Control experiments showed that the salts did not include any fermentable reducing substance before or after hydrolysis with acid.

The alcohol extract was evaporated to a small volume at a temperature of 35° while the pH was kept at about 6. Last traces of alcohol and water were removed *in vacuo*. The solids were dissolved in water and made up to 25 cc. After filtration one portion was hydrolyzed in N H_2SO_4 for 4 hours at 100° and the SO_4 removed with Ba. The remainder was analyzed for maltose and glucose as outlined below. The filtrates were free of glycogen and hexosemonophosphate, since these are removed by the treatment with heavy metal and Ba.

A high alkalinity copper-iodometric reagent and a heating period of 20 minutes was used in order to insure complete oxidation of such sugars as maltose. The reducing power of maltose with this copper reagent was found to be 54 % of that of glucose and hence a factor of 1.85 was used for conversion.

At an alkaline pH glucose but not maltose is fermented, while at an acid pH both sugars are removed by fermentation with (Fleischmann's) yeast. After trying out this method, which was kindly communicated to us by Dr. Somogyi, on known mixtures of glucose and maltose, it was applied to the muscle extracts.

In order to gain further information on the nature of the fer-

† 28 gm. $Fe_2(SO_4)_3$ + aq. dissolved in 100 cc. of 1.5 N H_2SO_4. After cooling below 10°, 34 gm. $HgSO_4$ are dissolved. (Private communication from Dr. E. S. West.)

mentable sugar in muscle, an aldose determination was carried out by means of the Willstätter-Schudel titration. An extract in which stimulated and unstimulated muscle were combined, showed 18.4 mg. % of fermentable aldose by means of the hypoiodide titration and 18.5 mg. % fermentable sugar by copper reduction. The close agreement between these 2 values indicates that fructose was not present and since mannose is only slowly fermented at an alkaline pH, the fermentable hexose present was most likely glucose.

The accompanying table shows the values obtained in mg. sugar per 100 gm. muscle. In spite of the vigorous treatment with heavy metal precipitants the extracts were free of non-fermentable reducing substances only in 2 instances. Experiment 1 shows that the combined treatment with Fe and Hg is more effective in removing non-fermentable reducing substances than treatment with Hg alone. The first precipitation with Fe+Hg removes the bulk of the non-fermentable reducing substances, while the second precipitation removes only small amounts.

TABLE I.

	Unhydrolyzed				Hydrolyzed		
Tissue	Total Reduction	Non-Fermentable	Maltose	Glucose	Total Reduction	Non-Fermentable	Plasma Sugar
Gastrocnemius*	22	8	5	11	34	5	96
Gastrocnemius†	51	19‡	6	29	82	29	
Gastrocnemius*	20	4	2	15	15	0	90
Gastrocnemius†	37	4	1	33	37	0	
Gastrocnemius*	12	0	0	12	9	0	112
Gastrocnemius†	25	0	0	25	29	0	
Gastrocnemius*	18	7§	2	10			102
Gastrocnemius†	29	6§	0	23			
Heart Ventricle	34	0		34	45	0	
Heart Ventricle	43	13§	0	30			

* Resting muscle.
† Stimulated muscle.
‡ $HgSO_4$ used instead of $Fe_2(SO_4)_3 + HgSO_4$.
§ One precipitation with Hg + Fe.

The average glucose content of the gastrocnemius at rest was 12 mg. % at an average plasma sugar level of 100 mg. %. This corresponds closely to the values obtained previously on rat muscle[1] at comparable plasma sugar levels. Significant amounts of a sugar behaving like maltose were found only in the first experiment and in this case acid hydrolysis caused an increase in fermentable sugar. In the other cases only traces of maltose were present and the fermentable sugar content after acid hydrolysis was practically the same as

that before hydrolysis. After short tetanic stimulation the glucose content of the gastrocnemius more than doubled, the average being 28 mg. %. Stimulation was, however, without effect on the maltose content of muscle or on the fermentable sugar content after acid hydrolysis. Apparently, intermediates between glycogen and glucose such as disaccharides do not accumulate in muscle as a result of stimulation. Heart muscle contained about as much glucose as stimulated skeletal muscle and did not contain an appreciable quantity of maltose.

Summary.—The increase in fermentable sugar content of muscle following tetanic stimulation is due to an accumulation of glucose. Maltose does not accumulate as a result of contraction.

7927 P

Cataract in Rats Fed on Galactose.

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A previous publication[1] reported the occurrence of cataractous changes in the eyes of all rats fed on rations containing lactose as the chief source of carbohydrate. Negative results with other carbohydrates tested led to an investigation of galactose as the next logical step. This sugar was fed to young rats at 35% and 25% levels corresponding to the galactose available from the 70% and 50% lactose rations fed in previous experiments.

Four rats on the 35% galactose ration developed mature bilateral cataract in 12, 14, 14, and 37 days respectively (average 19 days), whereas those on the 25% galactose ration were somewhat more delayed. The average time for the development of mature bilateral cataract in 49 rats fed the 70% lactose ration was 10 weeks, approximately 4 times as long. Controls fed on the 70% starch ration showed no eye changes.

The rations used in the experiment are shown in Table I.

Growth was subnormal on both galactose and lactose rations but galactose caused no diarrhea, a consistent result from lactose feeding. Galactosuria was more severe on galactose than on lactose rations. The calcium content of cataractous eyes was of the same

[1] Mitchell, H. A., and Dodge, W. M., *J. Nut.*, 1935, **37**, 37.

TABLE I.

	35% Galactose	25% Galactose	70% Lactose	70% Starch
Casein	15	15	15	15
Starch	35	45		70
Lactose			70	
Galactose	35	25		
Crisco	9	9	9	9
Salt Mixture	4	4	4	4
Cod Liver Oil	2	2	2	2

Brewers' yeast 0.2 gm. fed daily to all animals.

magnitude on the 2 sugars but the increase apparently took place in a shorter time in the rats fed galactose. The calcium content of all eyes showing mature cataract was 3 to 4 times that of eyes from older rats on the 70% starch ration.

TABLE II.
Calcium Content of Cataractous and Normal Eyes.*

Ration Group	Av. Age of Rats (days)	No. of Eyes Analyzed	Condition of the Eyes	Mg. Ca per 100 gm. of eyes (dry weight)
70% lactose	135	14	mature cataract	111.5
70% ''	175	19	'' ''	122.4
70% ''	125	14	immature ''	38.8
35% galactose	32	6	mature ''	105.0
70% starch	185	10	normal	32.1

*Calcium determinations were made by Dr. Helen Sternberger through the courtesy of Dr. Icie G. Macy of "The Children's Fund of Michigan."

A more complete and rapid absorption of galactose from the alimentary canal when the sugar was fed as such than when derived from hydrolytic cleavage, and a slow glycogenesis, may well account for the slightly high blood sugars and galactosuria noted in these rats. The latter finding would also indicate that some of the blood sugar was galactose. Kirby *et al.*[2] found the dextrose concentration of aqueous humor to be parallel to that of the blood. Assuming that galactose in the blood would also be found in the aqueous humor, the lens epithelium would be exposed not only to higher than normal sugar concentration but to the foreign sugar galactose. The work of Kirby *et al.*[3] on tissue cultures of lens epithelium *in vitro* has indicated in a striking manner that galactose is toxic to these cells in much lower concentrations than either glucose or fructose. If the capsular membrane undergoes even slight degenerative changes in the presence of galactose it is not difficult to conceive of an increased permeability of this membrane to the various inorganic

2 Kirby, D. B., and Wiener, R. v. E., *Trans. Am. Acad. Ophthalmology and Otolaryngology*, 1932, **37**, 165.

3 Kirby, D. B., Estey, K., and Weiner, R. v. E., *Trans. Am. Acad. Ophthalmology and Otolaryngology*, 1932, **37**, 196.

ions in the surrounding fluids, and a resulting disturbance in colloidal structure of the lens.

Cataract has been associated with parathyroid tetany and a low serum calcium resulting in a change of inorganic ions in the eye fluids which supposedly disturb the colloid equilibrium in the crystalline lens.

It would seem that the perfect transparency of the normal lens must be maintained by an extremely constant balance of inorganic ions and that any interference with the inorganic equilibrium might disturb the colloidal solution and cause some of the proteins of the lens to precipitate.

A number of questions arise as a result of these observations and hypotheses. What other blood and tissue changes are associated with lactose and galactose metabolism? What deficiencies or excesses, if any, will aggravate or hasten cataract development? Is there any food constituent or metabolic product which will protect the capsular membrane against sugar injury?

7928 P

Effect of Rapid Infusion on Venous Pressure: A Test of Cardiac Reserve.

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The response of the heart to increased work is an indication of its reserve strength. This response is measured clinically in various exercise tests, with observation of pulse and respiratory rate changes. These tests operate, at least in part, by increasing the return of venous blood to the heart. A similar mechanism is brought into action by increasing the volume of the venous return to the heart by injecting fluids intravenously. If such fluids are injected rapidly and in large volume, an increased load is put on the right side of the heart.

Since the introduction and widespread use of simple methods of infusion, there has been much discussion of the proper rate of injection of such fluids. In the investigation of this problem, observation of the venous pressure before, during and after the infusion offers the most direct physiological evidence of the ability of the

vascular system to accommodate the rapidly increased blood volume. The venous pressure measurements give a partial indication of the power of the heart to respond to increased load by increased work.

Venous pressures have been measured during infusions given in cases of diabetic acidosis and post-operative shock. Large volumes of fluid have been injected, up to 2,500 cc. of normal saline in one hour, without any alteration of the venous pressure from a normal level. (Table I.) In making these determinations a three-way

TABLE I.

Ma. Case No. 245481. Diabetic acidosis.

Min.	cc. of saline	Venous pressure mm. of water	Pulse	Arterial pressure
0	before infusion	75	92	112/54
5	200	55		
10	400	55		
15	600	57		
20	800	54		
25	1000	57		
30	1200	55		
35	1400	66		
40	1500	70		
45	1650	68		
50	1800	75		
55	2000	85		
60	2150	88	106	132/70
65	2350	87		
70	2500	87		140/80
—	——	—		
75		70		
80		52		146/78

stop-cock has been used connecting the needle in the vein with the infusion reservoir and the venous pressure apparatus (of Moritz and v. Tabora), and pressure readings have been taken at intervals of 2 to 5 minutes.

For extending this method in an attempt to develop an infusion test of cardiac function, the technique used has been to inject 1,500 cc. of normal saline solution into one of the arm veins in 30 minutes, with venous pressure values recorded at 2 minute intervals. In normal cases this has caused no alteration of the venous pressure level during or after the injection. In cases with known heart damage there has been a progressive rise in venous pressure during the injection, and very little tendency to return to the normal level when the injection has been stopped. (Table II.) In intermediate cases there has been a moderate rise in venous pressure during the injection, with a rather prompt fall to the normal level after the infusion.

More than 25 cases have been studied in this way.

TABLE II.

Pr. Case No. 438048. Hypertensive cardiovascular disease.

Min.	cc. of saline	Venous pressure mm. of water	Pulse	Arterial pressure
0	before infusion	70	72	
2		65	76	140/80
4		63	72	
6		70	72	
8		82	68	
10	550	83	68	145/80
12		92	72	
14		102	68	
16		108	68	150/82
18	1000	112	68	
20		117	72	
22		123	68	152/88
24		122	68	
26	1500	135	68	
—	—	—	—	
28		120	64	160/95
30		120	68	
32		115	64	
34		110	64	164/95
36		110	68	

From these observations the following suggestions are made: 1. Repeated venous pressure readings during an infusion give a measure of the ability of the vascular system to tolerate the increased volume of blood. 2. Cases of diabetic acidosis and postoperative shock may be given large amounts of fluid intravenously without elevation of the venous pressure. 3. Further work is necessary to show whether a useful test of cardiac function can be developed by measuring the reaction of the venous pressure during and after the intravenous injection of a large amount of fluid in a measured time interval.

7929 P

Viability and Virulence of Frozen and Dried Cultures of Meningococcus.

GEOFFREY RAKE

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One of the difficulties and time-consuming labors in work with the meningococcus has been the maintenance of cultures. Even with Hitchen's semi-solid ascitic fluid agar it is advisable to transfer

cultures every month and in no case can they be left much over 2 months. With other media the strains remain alive for much shorter periods. Bound up with this poor viability of meningococcus cultures in artificial media is the great difficulty of sending or transporting cultures on journeys requiring several days and exposure to varying temperatures. Cultures sent for example to Australia or India from this country almost never survive. At the same time it has been found that cultures lose certain of their characteristics on passage in artificial media. Thus the formation of the type-specific polysaccharide decreases[1] and, as the recent development of a mouse virulence test by Miller[2] has allowed one to show, the intraperitoneal virulence of a fresh culture may be rapidly lost during a few subcultures.

In order to obviate these difficulties, cultures of meningococci have been frozen and dried in our laboratory. Sixteen-hour cultures of the organisms on 10% rabbit's blood pneumococcus agar plates have been washed off with 10 cc. of hormone broth and distributed in 1 cc. amounts in soft glass tubes. These 1 cc. amounts have been rapidly frozen by immersion in a 95% alcohol-solid carbon dioxide snow mixture. The tubes are then placed in a high vacuum refrigerator and dried at —4°C. for 48 hours. At the end of this time the tubes are removed and placed in a desiccator at room temperature for 3 hours. They are then sealed off. It will be noted that as yet no attempt has been made to seal off *in vacuo*. This is shortly to be undertaken.

Tubes of frozen and dried material have now been prepared from 6 strains of meningococcus. Of these, 2 are old stock strains, and 4 are fresh strains. The 2 stock strains were still viable when last tested, that is at the end of 89 days. The 4 fresh strains were also viable when last tested, that is at the end of 151 days, 141 days, 89 days and 41 days respectively. One fresh strain, 520 M6, had an intraperitoneal virulence for mice at the time it was frozen and dried of $10^{-6}(+)$. Forty-one days after being frozen and dried this virulence was maintained.

It seems clear from this that both freshly isolated and stock strains of meningococci retain their viability when frozen and dried for many months. There is an indication also that the virulence of the cultures is maintained in the frozen and dried state at least over a period of 6 weeks. The technique of freezing and drying meningococcus cultures may be of use in storing cultures in a state approx-

[1] Rake, G., *J. Exp. Med.*, 1933, **58**, 361.
[2] Miller, C. P., *Science*, 1933, **78**, 340.

imating that of the strain when freshly isolated, and also form an answer to the problem as to how such delicate organisms shall be sent or transported over long distances where subculturing is impossible.

7930 P

Phenol Red Clearances in the Dog.

JAMES A. SHANNON. (Introduced by H. W. Smith.)

From the Department of Physiology, New York University, College of Medicine.

Marshall and his coworkers[1] were the first to adduce evidence that phenol red is excreted by the dog's kidney by tubular secretion as well as by glomerular filtration. This evidence was: (1) phenol red injected intravenously accumulated in the cells of the convoluted tubules of the anuric kidney obtained by spinal transection; (2) phenol red is in part bound to plasma colloids and thus rendered non-filtrable, and an insufficient concentration of filtrable phenol red is present in arterial blood to account for the quantity excreted in the urine; (3) in two experiments on anesthetized dogs the rate of excretion of phenol red was not proportional to the concentration in the plasma at all levels of the latter; (4) phenol red clearances in a normal dog were considerably greater than simultaneous creatinine clearances. The last mentioned experiments were done within a restricted range of plasma phenol red (0.21 to 0.54 mg. %) and leave undetermined the question of the relationship of the latter to the phenol red clearance, as well as the relationship of this clearance to the clearances of other urinary constituents.

Simultaneous phenol red and inulin clearances* have been determined in normal dogs, with special reference to the effect of the plasma concentration on the former. The results obtained upon one dog are illustrated in Fig. 1.

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1 Marshall, E. K., Jr., and Vickers, J. L., *Bull. Johns Hopkins Hosp.*, 1923, **36**, 1; Marshall, E. K., Jr., and Crane, M. M., *Am. J. Phys.*, 1924, **70**, 465; Marshall, E. K., Jr., *Am. J. Phys.*, 1931, **99**, 77.

* The use of inulin in renal studies has been discussed by Richards, Westfall, and Bott (PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 73); in the dogfish by Shannon (*J. Cell. Comp. Physiol.*, 1934, **5**, 301), and a second by communication by Shannon dealing with the excretion of inulin in the dog is now in press in the *American Journal of Physiology.*

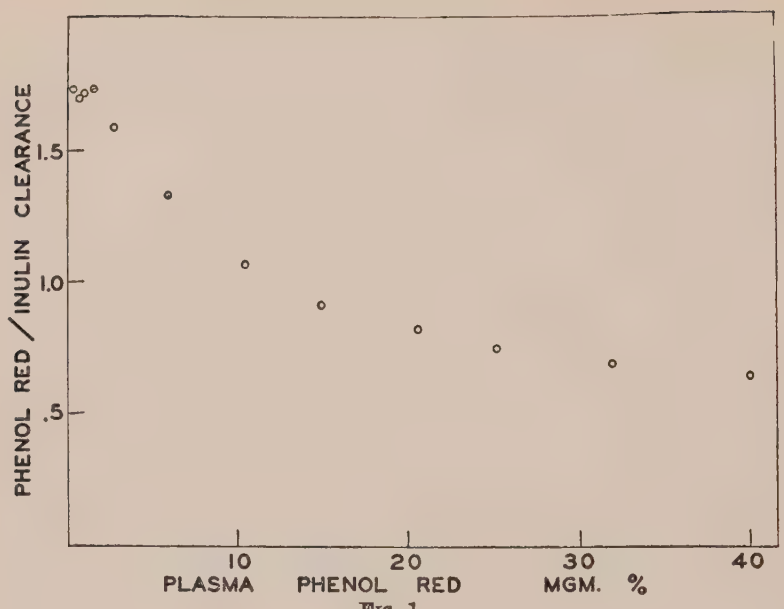

FIG. 1.

At plasma concentrations of phenol red from 3.0 to 2.0 mg. % down, the clearance of this substance (calculated upon the total dye in the plasma) has an approximately steady level, and the phenol red/inulin clearance ratio consequently is constant, having a value of about 1.7. As the plasma level of phenol red is increased, the phenol red clearance falls both absolutely and relative to the inulin clearance, until at a plasma level of 40 mg. % it is only 65% of the latter. Only 45% of the total phenol red in this experiment was filtrable through collodion at 38 mm. p CO_2 and 37°C., at this high level, and it is presumed that could the plasma level of dye be raised to a sufficient degree, the phenol red clearance, calculated on the basis of free phenol red, would approach the inulin clearance as a limiting value. The relationship between plasma level and clearance illustrated in the accompanying figure represents a reversible phenomenon, *i. e.*, the same clearance is observed at a particular plasma level whether the latter is reached on a rising or falling curve, or has been constant. Phlorizin has no appreciable effect upon the phenol red/inulin ratio.

7931 P

Phenol Red Clearances in Man.

WILLIAM GOLDRING, ROBERT W. CLARKE AND C. WELSH. (Introduced by H. W. Smith.)

From the Department of Physiology, New York University, College of Medicine, and the Medical Service, Third (New York University) Medical Division of Bellevue Hospital, New York City.

Although phenol red, introduced by Rowntree and Geraghty in 1912, has been widely used as an empirical renal function test in man, there is no information on the excretion of this substance in relation to the simultaneous excretion of other urine constituents.* With knowledge available concerning the order of magnitude of glomerular filtration in man,[1] a determination of phenol red clearance is of particular interest in indicating the relative rôle played by filtration and secretion in the excretion of this substance.

Preliminary observations following oral, intramuscular and intravenous administration of phenol red indicate that the latter is most suitable for investigative purposes. In the observations reported here, a sterile 10% solution of the dye, prepared for us by Hynson, Westcott and Dunning, was administered intravenously following the intravenous administration of inulin, and the experiments were so conducted that the simultaneous clearances of inulin and phenol red could be determined at various plasma levels of the latter, standard (basal) physiological conditions being maintained throughout. The dose of phenol red was varied from 300 mg. to 5 gm. per man, and observations were made at plasma concentrations ranging from 0.1 to 28.2 mg. %.

At plasma levels of the dye below 1.5 mg. %, the phenol red clearance is essentially constant, having an average value of about 400 cc. per minute, when calculated upon the total dye in the plasma. The phenol red/inulin clearance ratio averages about 3.2 under these conditions. A series of such observations on several individuals are recorded in the upper right hand corner of Fig. 1. As the plasma level of the dye is increased, the phenol red clearance is lowered both absolutely and relative to the inulin clearance, until the phenol red/inulin clearance ratio may be less than 1.00. This depression of the phenol red clearance appears to be reversible, the

* MacKay, E. M., (PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1039) has reported three observations on the relative ratio of excretion of phenol red and urea in man.

1 Shannon, J. A., and Smith, H. W., *J. Clin. Invest.*, in press.

same results being obtained, whether an intermediate plasma concentration is reached by the administration of a small dose, or on a falling plasma curve after the administration of a large dose. Results obtained in two series of observations on one individual (L.R.) are recorded in the figure.

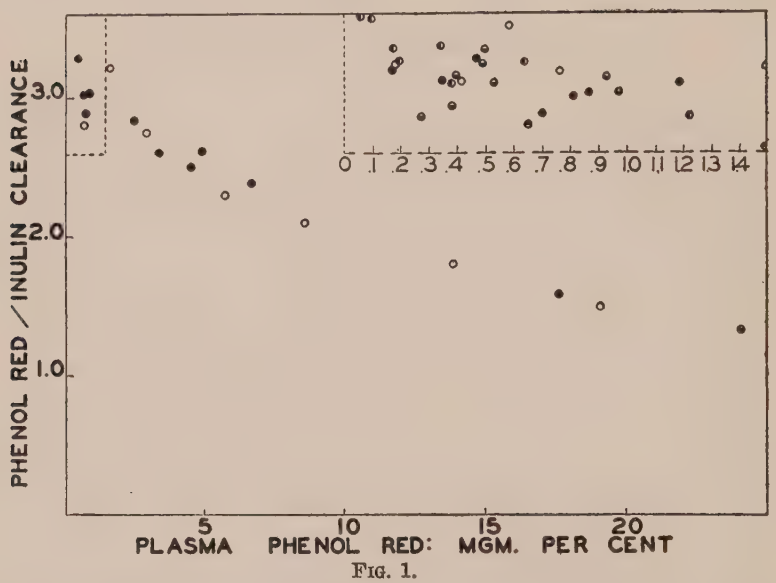

FIG. 1.

The magnitude of the phenol red/inulin clearance ratio, as well as the curvilinear relationship between plasma concentration and rate of excretion, indicates that the greater proportion of the dye is excreted by a process of tubular secretion. At 2.5 mg. % in L.R.'s plasma (35 mm. p_{CO_2} and 37.5°C.), 23% of the dye was filtrable through collodion. This figure is probably slightly less at lower plasma levels, but granting that filtration cannot account for the excretion, relative to the inulin clearance, of a greater fraction of the total plasma dye than this, it follows that about 8% of the dye appearing in the urine is excreted by filtration and 92% is removed from the post-glomerular blood and transferred to the tubular urine by tubular activity. At very low plasma levels (such as are obtained with the routine phenol red test) the fraction of dye excreted by tubular activity must be at least this large. If phenol red were excreted solely by filtration there would be required, when allowance is made for the small fraction of filtrable dye, 2000 cc. of filtrate per minute, which is considerably in excess of the probable blood flow to the kidneys.

The relations existing in man between plasma level and rate of excretion are entirely similar to those described in the dog by Shannon in the accompanying paper, except that at all plasma levels the phenol red clearance in man is relatively much greater than the inulin clearance. In man the maximal phenol red/inulin clearance ratio is 3.2 as compared to 1.7 in the dog. When one takes into account the fact that there is about twice as much free phenol red in dog's plasma as there is in man's, this difference becomes all the more significant in indicating a great difference in the capacity of the two kidneys to secrete this substance.

7932 C

Effect of Splenectomy on Bacterium Enteritidis Infection in White Mice.

JESSIE MARMORSTON

From the Department of Pathology of Cornell Medical College, New York City.

It has been well established that removal of the spleen in many species of animals depresses the natural resistance to certain bacterial, protozoan and spirochetal infections.* The rôle of the spleen in the maintenance of an acquired resistance to latent piroplasmidae and bacterial infections has been demonstrated in *Bartonella muris* infection in the rat, in *Bartonella canis* infection in the dog, in *Eperythrozoan coccoides* and *Bartonella muris* infection in the mouse, in infection with *Nuttalia equi* in the horse, Anaplasmosis in sheep and cattle and *Babesia bigeminum* in cattle.[3] Removal of the spleen converts a latent infection into manifest disease, often associated with a severe fatal anemia.

Morris and Bullock studied a spontaneous epizootic infection, with *Bacterium enteritidis* (Gaertner), in their rat colony and noted that splenectomy conspicuously lowers the resistance of the rat to this natural infection.[4] They were unaware, however, of the occurrence

* The relation of the spleen to resistance has been discussed in papers of Perla and Marmorston,[1] Marmorston[2], and in a recent monograph.[3]

1 Perla, D., and Marmorston-Gottesman, J., *J. Exp. Med.*, 1930, **52**, 601; 1931, **53**, 869, 877.

2 Marmorston, J., *J. Inf. Dis.*, in press.

3 Perla, D., and Marmorston, J., *The Spleen and Resistance*, Williams and Wilkins, Baltimore, 1935.

4 Morris, D., and Bullock, F., *Ann. Surg.*, 1919, **70**, 513.

of *Bartonella muris* anemia following removal of the spleen in almost all strains of rats.

In the experiments reported in this communication the effect of splenectomy on the course of a subsequently induced acute bacterial infection, *Bacterium enteritidis,* was studied in mice free of *Eperythrozoan coccoides* and *Bartonella muris,* since latent infections with these microorganisms may become active following removal of the spleen.[2]

All the mice used in these experiments were kindly furnished to us by Dr. Leslie T. Webster of the Rockefeller Institute for Medical Research. Two strains of mice were used. The animals of one strain were of a selected stock which had been found in the studies of Dr. Webster to be highly resistant (termed by him, "resistant") to spontaneous or induced infection with *Bacterium enteritidis* and pneumococcus. The mice of the other group (termed by him, "susceptible") were of a strain highly susceptible to these infections.[5] All of the mice were approximately 3 to 4 months of age. To confirm the absence of *Eperythrozoan coccoides* and of *Bartonella muris* in the mice, smears of the peripheral blood in the control and splenectomized mice were examined at intervals of one or 2 days during the entire experimental period of one month. These mice were found to be entirely free of these infections. At the end of the experiment all the mice were autopsied and microscopic sections of the kidneys were carefully examined for the presence of *Klossiella muris,* since the author had noted an increase in severity of *Klossiella muris* infection in young adult mice of certain strains following splenectomy. The strains of mice used in these studies were found to be free of this microorganism.

Effect of Splenectomy on the Course of Bacterium Enteritidis Infection in a Resistant Strain of Mice. The effect of splenectomy on a subsequently induced infection with *Bacterium enteritidis* in resistant mice was observed in 2 experiments. In the first experiment 45 mice were divided into 3 groups. Fifteen were splenectomized, in 15 a laparotomy was performed and 15 were used as controls. Six days after operation all were injected intraperitoneally with 0.3 cc. of an 18-hour broth culture of *Bacterium enteritidis.* Of the splenectomized mice 5 or 33% died within a week and only one control succumbed. (Table I.)

In the second experiment 77 mice were divided into 3 groups: 32 were splenectomized, in 20 a laparotomy was performed, and 25 were used as normal controls. Forty-eight hours after the opera-

[5] Webster, L. T., *J. Exp. Med.*, 1933, **57**, 793.

TABLE I.

Effect of Splenectomy in Mice of a Resistant Strain on Course of a Subsequently Induced Infection with *Bacterium enteritidis*.

A. Intraperitoneal Injection.

	No. Mice	No. Died	% Died
Splenectomy	15	5	33
Laparotomy	15	0	0
Controls	15	1	6

B. Intrastomachal Injection.

	No. Mice	No. Died	% Died	Mice That Had Positive Blood Cultures and Survived	
				No.	%
Splenectomy	32	9	28	19	79
Laparotomy	20	0	0	9	45
Controls	25	2	8	12	52

tion all received 0.5 cc. of an 18-hour broth culture of *Bacterium enteritidis* administered directly into the stomach through a fine catheter.† Cultures on eosin-methylene-blue media of blood drawn from the tail vein were made daily during a period of one month. Colonies were identified by subcultural methods on Russell's triple sugar media and by subsequent agglutination tests with immune serum against *Bacterium enteritidis* for differentiation from *B. aertrycke* (mouse typhoid).

With the dosage of organisms employed less than 4% of normal control mice of this strain and those in which a laparotomy was performed, succumbed to the infection within a month. Only 2 of the normal controls died of the infection within the experimental period. This agrees with the previous findings of Webster.[5] Of the 32 splenectomized mice, 9 or 28% died within 2 weeks after introduction of the bacteria. None of the mice in which a laparotomy had been performed succumbed. (Table I.)

The greatest number of mice in which positive blood cultures were obtained occurred in the splenectomized group. Of 23 splenectomized mice that survived the injection about 80% had one or more positive blood cultures. Of the 20 mice in which a laparotomy was performed 45% had one or more positive blood cultures. Of the 23 mice in which no operation was done 52% had one or more positive blood cultures during the period of the experiment. (Table I.) The greatest number of positive blood cultures in all the groups occurred on the fifth day. However, in those mice that survived the infection, persistently positive blood cultures were found

†I am grateful to Dr. L. Webster of the Rockefeller Institute for carrying out this procedure.

during the 2nd and 3rd weeks, in the splenectomized mice in greater numbers than in the control mice. There is little difference in the curves (Fig. 1) of the normal controls and in those of the laparotomy controls. After the 21st day, the blood cultures of all the surviving mice were negative.

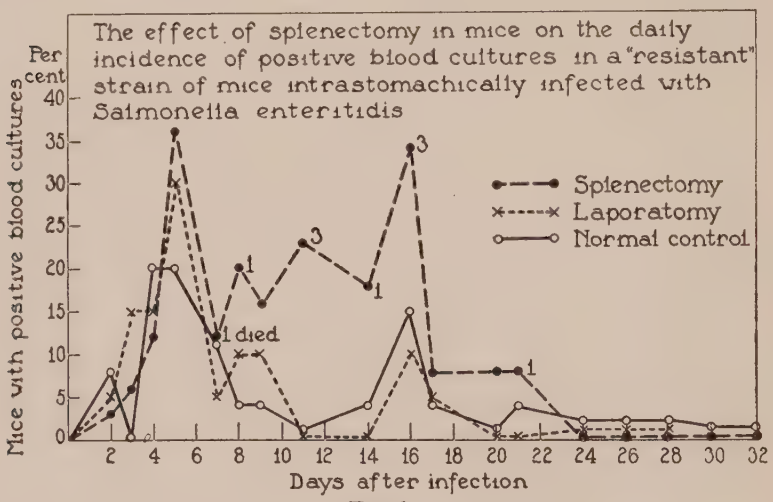

FIG. 1.

Effect of Splenectomy on the Course of Bacterium Enteritidis Infection in a "Susceptible" Strain of Mice. Twenty-three mice of stock highly "susceptible" to *Bacterium enteritidis* were divided into 3 groups (Table II). Splenectomy was done in 9, a laparotomy in 5, and 9 were used as controls. They were all infected by injection into the stomach, as in the previous experiments, 48 hours after operation. All animals, in which a laparotomy was performed, died. In the susceptible group the unoperated infected animals all developed bacteremia (see Fig. II) and 7 of 9 succumbed. There was a slightly higher mortality in the splenectomized than in the normal mice, but the experiment shows no significant difference between splenectomized and the 2 control groups.

In mice that are highly resistant to *Bacterium enteritidis* infec-

TABLE II.

Effect of Splenectomy in Mice of a Susceptible Strain on Course of a Subsequently Induced Infection with *Bacterium enteritidis.*

Operation	No. Mice	No. Died	% Died
Splenectomy	9	8	89
Laparotomy	5	5	100
Controls	9	7	77

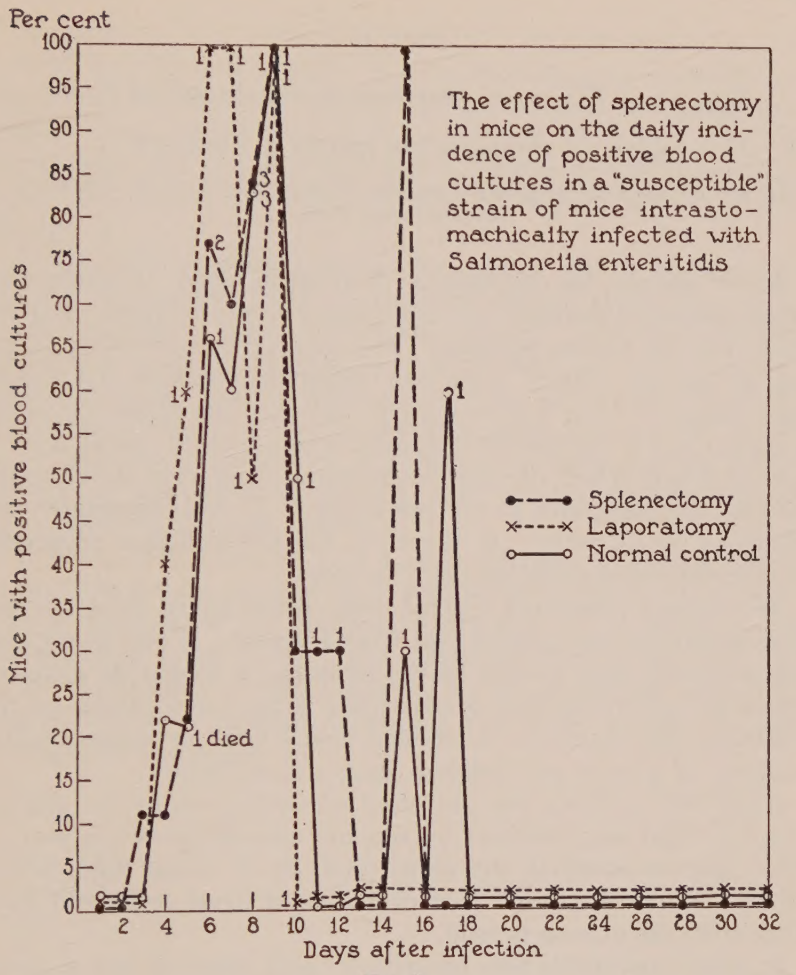

FIG. 2.

tion, the spleen has an important part in the maintenance of natural resistance to the acute infection. In mice of susceptible strains, which normally succumb in high percentages to enteritidis infection, the spleen offers little protection against the infection.

Summary. 1. In a strain of mice highly resistant to bacterial infection the removal of the spleen depresses the natural resistance to a subsequently induced infection with *Bacterium enteritidis.* 2. In a strain of mice highly susceptible to bacterial infection the removal of the spleen does not affect the natural resistance to a subsequently induced infection with *Bacterium enteritidis.*

7933 P

Resorcinol (Fructose) Reaction in Cerebrospinal Fluid.

ROGER S. HUBBARD AND HELEN R. GARBUTT.

From the Buffalo General Hospital and the Medical School of the University of Buffalo, New York.

Roe's[1] method for determining fructose in blood, an application of the resorcinol method, has been applied to 39 specimens of spinal fluid from 34 human subjects. The average concentration found was 4.1 mg. per 100 cc., while determinations on blood and plasma gave values of from 1 to 2 mg. per 100 cc. The substance which gives the test is completely destroyed when 2 cc. of spinal fluid is incubated with 0.5 cc. of yeast for 10 minutes, or when sterile spinal fluid is inoculated with *B. coli communis* or *B. coli communior* and incubated for 24 hours. It, therefore, has the biological properties of fructose and glucose, but not of sucrose.

The orcinol compound formed from dilute spinal fluid has the same absorption spectrum as that from fructose. When strong glucose solutions were treated by the technique a somewhat different spectrum, with relatively less absorption in the green portion of the spectrum, was obtained. Undiluted spinal fluid, or corresponding mixtures of glucose and fructose, gave similar spectra.

Additional evidence that the color was not given by the glucose in spinal fluid was obtained by repeated simultaneous analyses of dilute glucose solutions and serial dilutions of spinal fluid, which showed that only a small part of the color developed could have come from the glucose present.

It seems improbable that phosphoric acid esters of sugars could explain the finding, for (1) there is not sufficient organic phosphorus in spinal fluid to account for more than a trace of the "fructose" found,[2] and (2) when the method of Cori and Cori[3] was applied to spinal fluid only a small fraction—approximately 2%—of the total color-producing substance was precipitated.

The amount of "fructose" in the spinal fluid closely paralleled the amount of glucose (total reducing substances) present. It could be increased by injecting glucose. It was absent from a specimen containing no sugar, but practically free from cells.

It seemed logical to investigate the production of "fructose" from

[1] Roe, J. H., *J. Biol. Chem.*, 1934, **107**, 15.

[2] Youngburg, G. E., *J. Lab. Clin. Med.*, 1927, **12**, 845.

[3] Cori, G. T., and Cori, C. F., *J. Biol. Chem.*, 1931, **94**, 561.

dilute solutions of glucose (0.02 to 0.1%) by treatment with alkali. 0.15 M Na_2CO_3 and stronger alkaline solutions gave a marked production of the sugar on incubation, but there was no change when weaker alkalies (0.15 M Na_2HPO_4 or 0.15 M $NaHCO_3$) were used, except when the solutions were boiled. Boiling neutral glucose solutions also caused a marked increase in the amount of fructose present.

If the color-yielding substance in spinal fluid is formed from glucose, there must, therefore, be some factor besides the alkalinity of the solution taking part in the change. Some increase in the apparent amount of the compound giving the color was observed in each of 3 experiments in which glucose and sterile spinal fluid were incubated together, but the increase was slight (about 1 mg. per 100 cc. of solution) and close to the limit of accuracy of the method. Incubation of sterile spinal fluid by itself did not cause any increase in its apparent fructose content.

It seems to the authors that a rearrangement of glucose similar to that which takes place in dilute alkaline solutions is a fairly probable explanation of the presence of fructose, or of a fructose-like compound in spinal fluid. Such a rearrangement might take place in the fluid itself, as was suggested by the incubation experiment just described, or it might take place during the passage of sugar through the meninges.